7-2020

The Effects of Excess Dietary Sulfur Using Supplemental Sodium Sulfate on Beef Steer, Heifer, and Progeny Productive and Physiological Responses

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The Effects of Excess Dietary Sulfur Using Supplemental Sodium Sulfate on Beef Steer, Heifer, and Progeny Productive and Physiological Responses

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Animal Science

by

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July 2020
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ABSTRACT

For studies 1 through 3, 20 steers of predominantly Angus breeding, were stratified by body weight (279 ± 13.2 kg), assigned randomly to 6 paddocks, and fed a low S ground corn and soybean meal supplement that did not contain any byproduct feeds (0.31% total dietary S; **LS**) or LS supplement with an additional 0.25% S provided from sodium sulfate (**Na_2SO_4**; 0.58% total dietary S; **HS**) for a 114-d growing phase. Steers were moved to feedlot (373 ± 0.2 kg), remained on prior dietary S treatments, and fed corn and soybean meal diets (0.19 and 0.42% total dietary S; LS and HS treatments, respectively) that did not contain any byproduct feeds with no use of growth-enhancing technologies for a 123-d finishing phase. Steer performance was not affected ($P \geq 0.68$) by dietary treatments during the growing phase. Steers fed HS experienced decreased dry matter intake ($P < 0.001$) and average daily gain ($P = 0.07$) during the finishing phase; however, on a carcass-adjusted basis, dietary treatments did not affect average daily gain ($P = 0.24$). Steers fed HS produced longissimus muscle (**LM**) with greater ($P \leq 0.08$) total conjugated linoleic acid and 18:2*cis*9*trans*11 content than LM from steers fed LS; however, total conjugated linoleic acid content decreased ($P = 0.09$) during 7-d of simulated retail display. Finishing phase plasma Cu concentrations were less ($P = 0.07$) in steers fed HS than steers fed LS; however, plasma Cu concentrations were within the normal range and did not approach concentrations indicative of deficiency. Sulfhemoglobin production was greater ($P < 0.001$) in steers fed HS than steers fed LS (0.45 and 0.37%, respectively). Cytochrome *c* oxidase activity in liver and LM was not affected ($P \geq 0.38$) by dietary treatment. For study 4, 36 primiparous beef heifers (20 ± 0.5 mo of age) of predominantly Angus breeding were stratified by body weight (398 ± 24.9 kg), body condition score, and anticipated calving date and assigned to 12 paddocks for a 260-d study. Pens were assigned randomly to 1 of 4 treatments (2 × 2 factorial): 1) 0.15% S and 6 mg

Cu/kg; 2) 0.15% S and 12 to 14 mg Cu/kg (from tribasic copper chloride); 3) 0.55% S (from Na_2SO_4) and 6 mg Cu/kg; or 4) 0.55% S (from Na_2SO_4) and 12 to 14 mg Cu/kg (from tribasic copper chloride). A cracked corn and soybean meal based supplement delivered each treatment - 113 to 150 ± 16 d relative to parturition. Heifer growth performance was not affected ($P \geq 0.19$) by dietary treatments. Progeny from heifers fed 0.15% S and 6 mg Cu/kg exhibited lower birth weights (Cu × S interaction, $P = 0.09$); however, treatments did not affect ($P \geq 0.13$) other measures of progeny growth performance. Heifers fed 0.55% S exhibited lower plasma and liver Cu concentrations and plasma ceruloplasmin activity (S main effect, $P \leq 0.07$). Progeny liver Cu concentrations were similar among treatments and indicative of adequate liver Cu status in cattle, which was in contrast to maternal Cu indices.

ACKNOWLEDGMENTS

Appreciation is expressed to the members of my dissertation committee (Dr. Elizabeth Kegley, Dr. Jason Apple, Dr. Walter Bottje, and Dr. Jeremy Powell) for their constructive feedback, expertise, support, and suggestions that were essential to the successful completion of this work. Appreciation is also expressed to University of Arkansas Stocker Cattle Receiving and Backgrounding Unit [Pete Hornsby (retired) and Jana Reynolds], Meat Science (Dr. Janeal Yancey), and Ruminant Nutrition Laboratory (Doug Galloway and Karen Anschutz) staff for their assistance, knowledge, patience, and training that further contributed to my graduate education.

DEDICATION

This dissertation is dedicated to John and Johnny James Hawley. Thank you for your unwavering encouragement, patience, and support throughout this endeavor.

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LIST OF ABBREVIATIONS

a*	Redness
ADF	Acid detergent fiber
ADG	Average daily gain
A:P	Acetate:propionate
b*	Yellowness
BCS	Body condition score
BH	Biohydrogenation
BW	Body weight
$CaSO_4$	Calcium sulfate
CIDR	Controlled intravaginal drug release
CLA	Conjugated linoleic acid
CP	Crude protein
CytOx	Cytochrome *c* oxidase
DDGS	Dried corn distillers grains with solubles
DG	Corn distillers grains
DGS	Corn distillers grains with solubles
DM	Dry matter
DMI	Dry matter intake
FA	Fatty acid
FAME	Fatty acid methyl ester
H_2	Hydrogen
H_2S	Hydrogen sulfide
H_2SO_4	Sulfuric acid
Hb	Hemoglobin
HCW	Hot carcass weight
HS	High sulfur
HS^-	Hydrosulfide
JAR	Just-about-right
L*	Lightness

LHM	Liver homogenization medium
LM	Longissimus muscle
LS	Low sulfur
MHM_1	Muscle homogenization medium 1
MHM_2	Muscle homogenization medium 2
Na_2SO_4	Sodium sulfate
NDF	Neutral detergent fiber
NEg	Net energy for gain
$(NH_4)_2SO_4$	Ammonium sulfate
PEM	Polioencephalomalacia
PUFA	Polyunsaturated fatty acid
RD	Retail display
ROS	Reactive oxygen species
RTS	Reproductive tract score
S^{2-}	Sulfide
SHb	Sulfhemoglobin
SO_4^{2-}	Sulfate
SQ	Subcutaneous fat
SRB	Sulfate reducing bacteria
TBARS	Thiobarbituric acid reactive substances
USFA	Unsaturated fatty acid
WBSF	Warner-Bratzler shear force
WDGS	Wet corn distillers grains with solubles

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relative to parturition on postpartum primiparous beef heifer reproductive performance.

CHAPTER I

REVIEW OF LITERATURE

Sulfur requirements for ruminants

Sulfur is an important mineral for the optimal growth and overall physiology of cattle. Sulfur is required by ruminal microorganisms to synthesize the S-containing amino acids methionine, cysteine, and cystine, as well as the B-vitamins thiamine and biotin (Spears et al., 1976; NRC, 2000). Methionine is required for initiating amino acid synthesis for nearly all eukaryotic proteins, while cysteine and cystine are crucial for protein metabolism (Goodrich and Garrett, 1986; Brosnan and Brosnan, 2006). The B-vitamins thiamin and biotin are required for decarboxylation reactions, and are functional in transketolase and carbon fixation in ruminal microorganism, respectively (Goodrich and Garrett, 1986). In addition, many enzymes require S for disulfide bond formation and enzyme activity (Goodrich and Garrett, 1986).

Inadequate dietary S compromises optimal microbial activity in the rumen and leads to anorexia, excessive salivation, weight loss, and poor growth performance (NRC, 2000). The S requirement for ruminants is not clear, but it is suggested to be 0.15% S of dry matter (**DM**; NRC, 2000) or between 0.18 and 0.24% S (NRC, 2005), regardless of S source, to support the adequate growth and production of beef cattle. A survey of consulting feedlot nutritionists indicated that 0.22% S of DM was the recommended average amount of dietary S in beef cattle finishing diets, whereas 0.15% S of DM was the recommended minimum amount (Vasconcelos and Galyean, 2007). Ruminants fed corn-based finishing diets are usually close to these recommendations, as corn grain provides 0.14% S of DM, primarily as the amino acid methionine (Stock et al., 1995). However, dietary S can be inadvertently introduced into the ruminant diet through a variety of sources. In situations where byproducts from corn dry- or wet-milling plants are also part of the dietary ingredients [e.g., dried distillers grains with solubles

(**DDGS**) and wet corn gluten feed], dietary S is usually in excess due to the high S content in these byproducts (Buckner et al., 2008a). The increased use of these byproducts has led to considerable research evaluating the consequences of dietary S level in ruminant diets. The maximum tolerable limit for S in beef cattle diets has been suggested to be 0.30% S of DM in diets containing greater than 85% concentrate, and 0.50% S of DM in diets containing greater than 40% forage (NRC, 2005). The Mineral Tolerance of Animals (NRC, 2005) defines the maximum tolerable limit as the dietary concentration that will not impair animal health or performance when fed for a set period of time. The concentration of dietary S that limits dry matter intake (**DMI**) and decreases cattle performance appears to be less than that which causes S-related polioencephalomalacia (**PEM**; Drewnoski et al., 2014). The maximum tolerable limit of dietary S is based on the prevention of poor cattle performance and the increased risk of S-related PEM, and not based on avoiding the negative effects of dietary S on the absorption of trace minerals such as Cu or Se (NRC, 2005). The concentration of dietary S that interferes with trace mineral absorption is likely less than that which decreases cattle performance; however, the effect of dietary S on trace mineral status may be more easily overcome via nutritional strategies than the negative effects of dietary S on DMI and performance (Drewnoski et al., 2014).

Dietary sources of S

Organic sources of dietary S (S-containing amino acids) are the primary S source for ruminants. The organic S sources can be found in several crops, forages, and grains (e.g., corn). However, increased S can be introduced into the ruminant diet through a variety of sources. High protein feedstuffs with greater concentration of S-containing amino acids (e.g., canola, soybean, linseed, and peanut meals) can contribute a significant amount of S to the diet (1.17, 0.48, 0.43, and 0.33% S of DM, respectively; NRC, 1996; Stock et al., 1995). High S concentrations can

also be introduced to the diet through a variety of organic and inorganic feedstuffs including high sulfate (**SO_4^{2-}**) water, molasses, dried whey, S salts {e.g., ammonium [**$(NH_4)_2SO_4$**], calcium [**$CaSO_4$**], or sodium sulfate [**Na_2SO_4**]}, elemental S, byproducts from corn wet-milling plants where S dioxide is used in the steeping process (e.g., corn gluten feed), or byproducts from corn dry-milling plants (e.g., ethanol production) where sulfuric acid (**H_2SO_4**) is used as a decontamination agent and to control pH during fermentation [e.g., corn distillers grains with solubles (**DGS**); Kerr et al., 2008; Erickson et al., 2010]. The S concentration in byproducts from corn dry-milling plants can vary considerably as there is no standard inclusion of H_2SO_4 during the industrial process and solubles from the process can be added at different inclusions back into the grain fraction. Buckner et al. (2011) measured DGS from 6 ethanol plants and found the S concentrations across plants ranged from 0.71 to 0.84% S of DM, and variability between loads from the same plant ranged from 3 to 13%. In addition, diet pH can vary considerably between S sources. Brasche et al. (2012) found that at the same dietary S concentration (0.50% S of DM), when comparing the effects of 5 commonly used dietary S sources on the potential for toxicity in cattle, $CaSO_4$ and Na_2SO_4 increased diet pH compared to condensed corn distillers solubles, DDGS, and H_2SO_4 (pH = 4.79 and 4.79 vs. 4.18, 4.08, and 3.93, respectively). The NRC (2005) recommends cattle consuming high concentrate diets should not drink water containing greater than 600 mg SO_4^{2-}/L, whereas cattle fed diets with at least 40% forage may be able to safely drink water containing 2,500 mg SO_4^{2-}/L. A survey of 498 water samples from 23 states found the SO_4^{2-} concentration ranged from 200 to 7,600 mg SO_4^{2-}/L (Gould et al., 2002). Sulfur contributions from water and feed ingredients are additive, thus both contributions should be used to determine the total S intake by ruminants.

Ruminal availability of S. While multiple sources of S can be metabolized in the rumen, ruminal S availability can vary depending on S source. Accordingly, total S intake may not equal the concentration of dietary S available for metabolism by ruminal sulfate reducing bacteria (**SRB**; Sarturi et al., 2013a). Greater microbial protein synthesis was observed after 18 h of incubation for organic (L-methionine) compared to inorganic [$(NH_4)_2SO_4$, $CaSO_4$, and Na_2SO_4] S sources in *in vitro* cultures using rumen fluid from lambs fed purified diets (Kahlon et al., 1975). Greater urinary excretion of labeled ^{35}S was detected for an inorganic (Na_2SO_4) compared to an organic (L-methionine) S source when lambs were fed a ground corn and corn silage pellet basal diet (Johnson et al., 1970). Greater S plasma concentrations at 9 and 24 h and whole blood S concentrations at 3 and 36 h for organic (DL-methionine) compared to inorganic (elemental S and Na_2SO_4) S sources were observed when heifers were fed a tall fescue hay-based diet (Fron et al., 1990). These results suggest that inorganic and organic S sources may not have the same bioavailability for ruminal metabolism by SRB. Inorganic S sources [e.g., $(NH_4)_2SO_4$, $CaSO_4$, and Na_2SO_4] are readily available for ruminal reduction by SRB. Conversely, organic S sources (e.g., S-containing amino acids in corn and DGS) are less available for ruminal reduction by SRB as some proportion of the dietary protein will escape degradation or be incorporated into rumen microbes. However, the excess S in DGS with that comes from the use of H_2SO_4 during ethanol production should be completely available for reduction by SRB.

Sulfur metabolism in ruminants

The reduction of SO_4^{2-} in the rumen is accomplished by assimilatory and dissimilatory SRB (Slyter et al., 1986; Kung et al., 1998). In assimilatory reduction, bacteria (*Bacteroides*, *Butyrivibrio*, and *Lachnospira*) reduce SO_4^{2-} to hydrogen sulfide (**H_2S**) for their own metabolic needs and to produce S-containing amino acids or co-factors such as biotin and pantothenic acid

(Emery et al., 1957; Cummings et al., 1995a; Cummings et al., 1995b; Bradley et al., 2011). In dissimilatory reduction, bacteria (*Desulfovibrio* and *Desulfotomaculum*) reduce SO_4^{2-} to H_2S for their energy needs, but produce more H_2S than needed (Cummings et al., 1995a; Cummings et al., 1995b; Bradley et al., 2011). Many SRB can also reduce other oxidized inorganic S compounds (sulfite, thiosulfate, or elemental S) as well as S-containing amino acids (Coleman, 1960; Barton and Fauque, 2009). Sulfate reducing bacteria comprise a small portion (less than 1%) of the ruminal bacterial population (Callaway et al., 2010), with dissimilatory outnumbering assimilatory SRB (Huisingh et al., 1974). As a result, the dissimilatory SO_4^{2-} reduction pathway is the predominant means of SO_4^{2-} reduction and accounts for the majority of the H_2S production in the rumen (Cummings et al., 1995a; Cummings et al., 1995b). It is believed that ruminal H_2S production is the primary cause of much of the detrimental effects of high dietary S on ruminant performance and health (Kandylis, 1984; Gould, 1998).

Ruminal H_2S production. The ruminal reduction of SO_4^{2-} (Fig. 1) proceeds with the formation of adenosylsulfate, sulfite, and sulfide ($\mathbf{S^{2-}}$; Russell, 2002). The concentration of S^{2-} in the rumen fluid is not static and is greatly affected by increasing dietary S and rumen pH (Fig. 2; Beauchamp et al., 1984). The acidic nature of the rumen favors H_2S production, with greater concentrations of H_2S in the ruminal gas cap when the rumen pH is less than 7.0 (Beauchamp et al., 1984; Kung et al., 1998; Schoonmaker and Beitz, 2012). With a change in ruminal pH from 6.8 to 5.2, H_2S in the ruminal gas cap increased from 46.8 to 97.2%, whereas hydrosulfide ($\mathbf{HS^-}$) in the rumen fluid decreased from 50.4 to 2.8% (Bray and Till, 1975). Gould et al. (1997) suggested 2,000 mg H_2S/L as the concentration at which cattle are at risk of developing S-related PEM. Increasing dietary S to 0.12, 0.30, and 0.37% in a ground corn-based finishing diet with Na_2SO_4 increased ruminal H_2S concentrations in lambs fed 0.30 and 0.37% S of DM (Felix et al.,

2014). However, ruminal H_2S concentrations peaked below the suggested 2,000 mg H_2S/L threshold at 1,131 and 1,495 mg H_2S/L for the lambs fed 0.30, and 0.37% S of DM, respectively. Pogge et al. (2016) fed steers differing inclusions of DDGS (20, 40, or 60% DM; 0.28, 0.41, or 0.56% S of DM, respectively) replacing dry-rolled corn. With increasing DDGS inclusion, H_2S in the ruminal gas cap increased (1,054, 3,155, and 4,298 mg H_2S/L; 0, 20, or 40% DDGS, respectively). Felix et al. (2014) reported that at the same dietary S concentration, acidic dietary S sources (i.e., 60% DDGS or corn-based diet with H_2SO_4) increased H_2S in the ruminal gas cap compared to a neutral dietary S source (i.e., Na_2SO_4). These results suggest that dietary S source may also be an important factor in the production of ruminal H_2S.

Ruminal H_2S mechanisms of toxicity and energy depletion. Hydrogen sulfide is a normal product of rumen microbial metabolism. However, episodes of excessive production and absorption of ruminal H_2S are a hazard to ruminants with important biological effects including sulfhemoglobinemia, S-related PEM, and energy depletion. Sulfhemoglobinemia is a condition in which there is excess sulfhemoglobin (**SHb**) in the blood. During occurrences of excessive ruminal H_2S production, H_2S can be absorbed across the rumen wall into the portal blood to oxidize the heme moiety in hemoglobin to form irreversible SHb, making functional hemoglobin incapable of carrying oxygen (Drabkin and Austin, 1935; Bulgin et al., 1996). Digesti and Weeth (1976) found increasing the total dietary S from 0.36 to 0.57% increased SHb concentrations in growing heifers when supplementing drinking water with Na_2SO_4 [110, 1,250, and 2,500 mg SO_4^{2-}/L; 0.20, 0.36, and 0.57% total (water plus feed) dietary S, respectively]. Similarly, Pogge and Hansen (2013) observed increasing dietary S (0.22, 0.34, or 0.55% S of DM) linearly increased rumen H_2S and blood SHb concentrations in feedlot steers.

Polioencephalomalacia is a neuropathological condition of ruminants that can be induced by a variety of neural metabolic disruptions (Gould, 1998). Investigations of S-related PEM have demonstrated that the onset of clinical signs coincides with excessive ruminal H_2S production (Gould et al., 1997; Loneragan et al., 2005). Two S-related PEM syndromes are recognized (Jensen et al., 1956). In one syndrome, signs occur acutely and animals are usually recumbent and comatose. These animals generally die due to irreparable brain damage. In the second syndrome, animals show clinical signs of central nervous disease over varying periods of time. Signs include ataxia, fine muscle tremors of the face and head, circling, head pressing, stupor, and cortical blindness. These signs may be followed by lateral recumbency, clonic-tonic convulsions with paddling motion, and death (Niles et al., 2002). Animals that have recovered may be unproductive due to permanent brain damage. Diagnostic confirmation of S-related PEM is made by the distribution and severity of malacia brain lesions (Hamlen et al., 1993; Jeffrey et al., 1994) and a distinctive autofluorescence under ultraviolet light in the cerebral cortex (Edwin and Jackman, 1981). The etiology of S-related PEM as a form of subacute H_2S toxicity is not yet fully understood. However, it is speculated that eructated H_2S gas is inhaled by the animal (Dougherty et al., 1962; Drewnoski et al., 2014; Fig. 3), bypasses detoxification in the liver, and enters the brain to cause necrosis of the grey matter (Gould et al., 1997). Dougherty et al. (1965) infused the rumen of sheep with H_2S gas and found those with open tracheas collapsed after several eructations, whereas those with an artificially blocked trachea showed no symptoms of toxicity. These results suggest that the mechanism of H_2S toxicity is not through the ruminal absorption of H_2S, but through the inhalation of eructated H_2S. The affinity of H_2S for the grey matter in the brain is attributed to its high lipid content (Olkowski et al., 1992) and high demand for oxygen (Rousseaux et al., 1991).

Subacute H_2S toxicity has also been attributed to the inhibition of mitochondrial Complex IV or cytochrome *c* oxidase (**CytOx**) activity (Petersen, 1977; Hill et al., 1984). Cytochrome *c* oxidase is the terminal membrane-bound enzyme in the mitochondrial electron transport chain. The enzyme is located in the inner mitochondrial membrane where it accepts electrons from reduced cytochrome *c* and brings about the 4-electron reduction of oxygen to water. A redox-linked translocation of protons across the inner mitochondrial membrane used for the generation of ATP is associated with this process. Accordingly, CytOx serves the dual role of generating a proton gradient by coupling redox events to proton translocation and maintaining the continued flow of electrons for oxidative phosphorylation by catalyzing the 4-electron reduction of oxygen to water. Hydrogen sulfide noncompetitively binds to CytOx to inhibit the reduction of oxygen to water (Petersen, 1977). Once the reduction of oxygen to water is inhibited, electron transport in the mitochondrial electron transport chain is disrupted, the potential of the mitochondrial inner membrane dissipates, and oxidative phosphorylation and generation of ATP is impaired. (Beauchamp et al., 1984). Toxicology studies focusing on inhalation H_2S exposure have established the inhibitory effect on CytOx activity in the lung and heart tissue of rats and its associated pathological alternations (Khan et al., 1990; Dorman et al., 2002, 2004; Wu et al., 2011). Conversely, while studies focusing on S-related PEM in cattle have established the pathological alternations associated with inhalation H_2S exposure, the inhibitory effect on CytOx activity has not been fully established. When replacing steam-flaked corn with wet corn distillers grains with solubles (**WDGS**; 0, 30, or 60% DM; 0.14, 0.26, or 0.41% S of DM, respectively), steers fed a 60% WDGS diet experienced greater ruminal H_2S concentrations compared to steers fed a 30% WDGS diet, but liver and muscle CytOx activity was consistent between treatments

(Ponce et al., 2014). However, brain tissue from steers fed the 60% WDGS diet tended to have lower CytOx activities compared to brain tissue from steers fed the 30% WDGS diet.

Effects of excess dietary S on ruminal metabolism

Ruminal carbohydrate-fermenting bacteria require the continuous removal of hydrogen (**H_2**) for sustained organic matter digestion and volatile fatty acid production (Sharp et al., 1998). On growing diets, methanogenic bacteria are primarily responsible for removing H_2 produced during microbial fermentation via methanogenesis. Methanogens are sensitive to ruminal pH with methanogenesis essentially absent at pH less than 6.0 (Van Kessel and Russell, 1996). As a result, on finishing diets, the drop in ruminal pH inhibits methanogens while carbohydrate-fermenting bacteria utilize propionate producing bacteria as an alternative mechanism for H_2 removal (Ellis et al., 2008). It is possible, however, that on a high S diet the number of SRB can readily surpass propionate producing bacteria to compete for available H_2 (Ellis et al., 2008). The addition of Na_2SO_4, a neutral dietary S source, to *in vitro* cultures (0.17 to 0.42% S of DM) using a steam-flaked corn substrate increased the molar proportion of propionate with a concomitant decrease in the acetate to propionate (**A:P**) ratio (Quinn et al., 2009). However, the adaptation of SRB to decreased ruminal pH and increased S concentrations from an acidic dietary S source [e.g., DDGS, WDGS, and $(NH_4)_2SO_4$] could potentially decrease molar proportions of propionate to a greater extent than a neutral dietary S source (e.g., Na_2SO_4). Accordingly, dietary S source may be an important factor in altering the molar proportions of volatile fatty acids produced by ruminal microorganisms. Sarturi et al. (2013b) observed steers fed a 1.16% S DDGS (40% DM; 0.54% S of DM) diet had lower molar proportions of propionate and tended to have a greater A:P ratio compared to steers fed 0.82% S DDGS (40% DM; 0.40% S of DM). Similarly, Luebbe et al. (2012) found molar proportions of acetate increased linearly whereas

molar proportions of propionate decreased linearly with increasing WDGS inclusion (15, 30, 45, or 60% DM; 0.22, 0.29, 0.36, or 0.44% S of DM, respectively). Conversely, when Zinn et al. (1997) fed steers differing inclusions of $(NH_4)_2SO_4$ (0.15, 0.20, and 0.25% S of DM) in a flaked corn-based finishing diet, the addition of $(NH_4)_2SO_4$ decreased molar proportions of acetate and increased molar proportions of propionate. The varying results reported by Zinn et al. (1997) and those by Sarturi et al. (2013b) and Luebbe et al. (2012) can likely be attributed to the dietary S concentrations evaluated. The dietary S concentrations evaluated in Zinn et al. (1997) may have been insufficient to change ruminal microbial population dynamics.

Effects of excess dietary S on ruminant performance

Recent research evaluating the consequences of dietary S level in growing and finishing ruminant diets have typically utilized acidic [e.g., H_2SO_4, various types of DGS, or $(NH_4)_2SO_4$] or a mixture of (e.g., DGS supplemented Na_2SO_4) dietary S sources, whereas the use of neutral (e.g., Na_2SO_4) dietary S sources has been limited. Regardless, the variable effects of supplemental S on ruminant performance are likely caused, in part, to the S source used and the corresponding change in dietary pH.

Growth performance. Digesti and Weeth (1976) observed that increased dietary S, when supplied in the drinking water, did not affect the performance of growing heifers. In their study, supplementing drinking water with Na_2SO_4 [110, 1,250, and 2,500 mg SO_4^{2-}/L; 0.20, 36, and 0.57% total (water plus feed) dietary S, respectively] to increase the total dietary S intake did not affect DMI or average daily gain (**ADG**). Conversely, Cammack et al. (2010) and Weeth and Capps (1972) observed increasing the total dietary S intake of growing cattle with supplemental Na_2SO_4 impacted performance. Cammack et al. (2010) administered drinking water supplemented with Na_2SO_4 [566 and 3,651 mg SO_4^{2-}/L; 0.28 and 0.79% total (water plus feed)

dietary S, respectively] to forage-fed steers. Increasing total dietary S from 0.28 to 0.79% decreased intake in the first 53 d of the study, but DMI was not affected by dietary S in the final 23 d of the study. Similarly, Weeth and Capps (1972) observed that increasing the total dietary S from 0.41 to 0.63% decreased the intake of growing heifers when supplementing drinking water with Na_2SO_4 [110, 1,462, and 2,814 mg SO_4^{2-}/L; 0.20, 0.41, and 0.63% total (water plus feed) dietary S, respectively]. Differences in growing cattle performance results when evaluating dietary S level using Na_2SO_4 may be attributed to differences in the level of dietary S evaluated and study duration. The greatest dietary S concentration in Weeth and Capps (1972) and Cammack et al. (2010) were 0.63 and 0.79%, respectively. Research indicates that SRB in the rumen require time to adapt to dietary S, especially when transitioning cattle to diets that exceed the maximum tolerable level of S (Drewnoski et al., 2012; Felix et al., 2014). Results from Cammack et al. (2010) suggest an adaptation of SRB in the rumen to excess dietary S. Increasing dietary S decreased intake in the first 53 d of the study, whereas intake was not affected by in the final 23 d of the study. However, when combining chronic exposure to increased dietary S with a 30-d trial, Weeth and Capps (1972) may not have allowed sufficient time for SRB to adapt to the increased ruminal SO_4^{2-} concentration.

Reported effects of increased dietary S from acidic dietary S sources on performance responses in growing cattle are variable. Increasing the S concentration in grasses from 0.22 to 0.51% with $(NH_4)_2SO_4$ fertilizer (Arthington et al., 2002) or from 0.12 to 0.46% in a corn silage-based growing diet with $(NH_4)_2SO_4$ (Spears et al., 2011) did not affect DMI in growing cattle. Conversely, Buttrey et al. (2012) found acidic dietary S source supplementation during the growing phase altered performance. Steers supplemented DDGS at 0.5% of BW (0.40% total dietary S) during the growing phase gained more and had greater final BW than steers provided

no supplement or supplemental dry rolled corn. Acidic ruminal pH can reduce the activity of cellulolytic and proteolytic bacteria to decrease dietary fiber and protein fermentation. Because $(NH_4)_2SO_4$ and DDGS are inherently acidic, it is conceivable that the S sources would decrease animal performance owing to reduced dietary fiber and protein digestion. However, the high-fiber diets likely promoted chewing and saliva production, to buffer ruminal pH and mitigate differences when comparing Na_2SO_4 with $(NH_4)_2SO_4$ and DDGS as dietary S sources in growing cattle diets. Positive responses may be attributed to the buffering capacity of the high-fiber diets combined with the high energy value of DDGS.

Finishing performance. Reported effects of increased dietary S from neutral dietary S sources on the performance responses in feedlot ruminants are limited. Felix et al. (2014) observed that increasing dietary S to 0.12, 0.30, and 0.37% in a ground corn-based finishing diet with Na_2SO_4 increased ADG and improved feed efficiency in lambs fed both 0.30 and 0.37% S. However, the potentially greater ruminal pH due to Na_2SO_4 and lasalocid supplementation likely caused an additive response to improve performance in their study.

Reduced feedlot performance is primarily observed with the increased inclusion of acidic dietary S sources. Bolsen et al. (1973) and Spears et al. (2011) observed decreased DMI when steers were fed $(NH_4)_2SO_4$ as a supplemental source of dietary S in finishing diets (0.41% and 0.47% S of DM, respectively). Felix et al. (2012) observed DMI linearly decrease with increasing levels of DDGS (0, 20, 40, or 60% DM; 0.11, 0.20, 0.31, or 0.43% S of DM, respectively) replacing rolled corn. When cattle were fed a 60% DDGS diet (0.43% S of DM), ADG and DMI decreased 16 and 13%, respectively, when compared to a 0% DDGS corn-based control diet (0.12% S of DM; Felix et al., 2014). Sarturi et al. (2013b) reported steers fed increasing levels of a 0.82% S DDGS (20, 30, or 40% DM; 0.26, 0.33, or 0.40% S of DM,

respectively) diet experienced a linear increase in DMI when compared to steers fed a 0% DDGS corn-based control (0.13% S of DM) diet. However, steers fed increasing levels of a 1.16% S DDGS (20, 30, or 40% DM; 0.33, 0.43, or 0.54% S of DM, respectively) diet experienced a quadratic decrease in DMI when compared to the same 0% DDGS corn-based control (0.13% S of DM) diet. It is conceivable the observed differences in finishing performance when supplementing various dietary S sources may reflect ruminal pH as ruminal H_2S concentration may increase when more hydrogen ions are available (i.e., acidic dietary S source) to form H_2S. Accordingly, finishing diets containing excess dietary S from neutral S sources may be less detrimental to ruminant performance when compared to diets containing excess dietary S from acidic S sources.

Carcass characteristics. Effects of increased dietary S from neutral dietary S sources on carcass characteristics have not been reported, whereas reported effects of increased dietary S from acidic dietary S sources on carcass characteristics have varied among studies. Spears et al. (2011) fed steers differing inclusions of $(NH_4)_2SO_4$ (0.13, 0.31, and 0.46% S of DM) in a corn-based finishing diet. Carcass characteristics did not differ among steers fed diets containing 0.13 and 0.31% S of DM, whereas steers fed diets containing 0.46% S of DM had decreased hot carcass weight (**HCW**), longissimus muscle area, quality, and yield grade compared to steers fed 0.13% S of DM. Increasing DDGS inclusion (20, 40, or 60% DM; 0.28, 0.41, or 0.56% S of DM, respectively) linearly decreased HCW and increased dressing percentage, whereas marbling scores showed a quadratic response with optimum inclusion at 40% (Pogge et al., 2016). Luebbe et al. (2012) reported HCW, 12th-rib fat thickness, marbling score, and calculated yield grade linearly decreased with increasing WDGS inclusion (15, 30, 45, or 60% DM; 0.22, 0.29, 0.36, or 0.44% S of DM, respectively). Buckner et al. (2008b) fed increasing levels of DDGS (0, 10, 20,

30, or 40% DM; 0.15, 0.24, 0.33, 0.41, or 0.55% S of DM, respectively) replacing dry-rolled corn and observed a quadratic response for HCW with optimum inclusion at 20%. Differences observed between results reported for those utilizing acidic dietary S sources could be attributed to starch availability and differences in extent of ruminal and post-ruminal starch digestion. Starch availability and differences in extent of ruminal and post-ruminal starch digestion have been reported to influence how DGS affect carcass characteristics. Corrigan et al. (2009) found increased corn processing (dry-rolled, high-moisture, or steam-flaked corn), when combined with increasing levels of WDGS (0, 15, 27.5, or 40% DM), affected carcass characteristics more severely at greater inclusion rates.

Effects of excess dietary S on beef quality, shelf-life, and fatty acid composition

When compared to feed ingredients such as corn, DGS contain a greater concentration of polyunsaturated fatty acids (**PUFA**). Greater PUFA deposition is regularly observed in muscular tissue when DGS are substituted for corn (Mello Jr. et al., 2012; Veracini et al., 2013; Domenech-Pérez et al., 2017; Ribeiro et al., 2018). It has been established that beef with greater concentrations of PUFA is more likely to exhibit increased lipid and myoglobin oxidation (Faustman et al., 2010). This is significant because lipid and myoglobin oxidation reduce beef color stability during retail display.

Lipid oxidation, as indicated by thiobarbituric acid reactive substances (**TBARS**) concentration, suggests that DGS impacts the TBARS concentration in beef. Buttrey et al. (2013) compared WDGS in steam-flaked corn based finishing diets at 0 or 30% of diet DM (0.12 and 0.34% S of DM, respectively) and WDGS in dry-rolled corn based finishing diets at 0 or 30% of diet DM (0.12 and 0.34% S of DM, respectively). Loin steaks from steers fed the 30% WDGS diet exhibited greater oxidation after 7 d of retail display compared to loin steaks from steers fed

the 0% WDGS diet. Conversely, others have reported no differences in TBARS in beef from cattle fed dry-rolled and steam-flaked corn-based diets containing 0 to 75% DGS of diet DM (Gill et al., 2008; Depenbusch et al., 2009; Gunn et al., 2009). These findings suggest that dietary S source may be an important characteristic that influences lipid oxidation. The reported effects of excess dietary S using Na_2SO_4 in cattle diets on beef shelf-life and FA composition are unknown. However, these results suggest that the greater PUFA content in beef from cattle fed DGS as a dietary S source will likely exhibit greater lipid oxidation and reduced color stability during retail display when compared to beef from cattle fed corn-based diets supplemented Na_2SO_4 as a dietary S source.

Effects of excess dietary S on fetal programming

Considerable research has evaluated the consequences of dietary S level from a variety of S sources in growing and finishing cattle diets, but the effects of dietary S level and S source on fetal programming are unknown. Fetal programming is the concept that a maternal stimulus or insult at a critical period in fetal development has long-term effects on the progeny (Funston et al., 2010). Trace minerals are essential for bovine fetal development, and the fetus depends completely on the dam for an adequate supply of these elements (Hidiroglou and Knipfel, 1981; Hostetler et al., 2003). If the maternal mineral supply is inadequate, newborn offspring may have low body reserves and are susceptible to trace mineral deficiencies and decreased performance early in life (Weiss et al., 1983).

The bovine fetus has a substantial demand for Cu. There is an increase in Cu deposition in fetal tissues throughout gestation (Moss et al., 1974; Graham et al., 1994), and Cu is required for the proper development of the fetal nervous, reproductive, and immune systems (Hostetler et al., 2003). The Cu antagonists S, Mo, and Fe induce Cu deficiency by forming insoluble Cu

complexes in the digestive tract, blood stream, and tissues of ruminants (Dick et al., 1975; Suttle and Field, 1983; Allen and Gawthorne, 1987). The reported effects of feeding Cu deficient diets supplemented the dietary antagonists Mo and Fe on the productive and physiological responses of beef heifers and their progeny are limited. Gengelbach et al. (1994) reported that feeding primiparous beef heifers Cu deficient diets with supplemental dietary Cu antagonists pre- and postpartum did not affect heifer growth performance. In their study, supplementing 5 mg Mo/kg or 600 mg Fe/kg of DM to a diet containing 4.0 mg Cu/kg, 0.32% S, 1.3 mg Mo/kg, and 192 mg Fe/kg of DM did not affect postpartum weight change. However, progeny from heifers fed elevated levels of Mo experienced decreased weight gain. There were no differences in progeny serum Cu concentrations due to treatment at approximately 7 d of age, and all treatment means for serum Cu were in the adequate range. However, by d 168 of the study (progeny between 43 and 98 d of age) and at all subsequent sampling dates, progeny from heifers fed elevated levels of Mo and Fe had decreased plasma Cu concentrations and ceruloplasmin activities consistent with Cu deficiency. Muehlenbein et al. (2001) reported no difference in the postpartum weight change of heifers, calf weaning weights, or in 60-d postpartum pregnancy rates; however, Cu deficiency in heifers and progeny was induced by supplementing the heifer's marginally Cu deficient diet with Fe and Mo. The effects of feeding Cu deficient diets supplemented S as a dietary antagonist on the productive and physiological responses of beef heifers and their progeny are unknown; however, these results suggest that maternal Cu deficiency due to the provision of Cu deficient diets and the Cu antagonist S may have the potential for programming progeny development.

Summary of implications from the literature review

The review of the literature suggests that the increased use of byproducts from corn dry- or wet-milling plants has led to considerable research evaluating the consequences of dietary S

level in growing and finishing ruminant diets; however, different sources of dietary S have been used. Accordingly, variability of the effects of added S to ruminant diets on ruminal metabolism, live and carcass performance, and meat quality among research trials is likely caused, in part, to the form of supplemental S used. Dietary S sources differ in their bioavailability for ruminal reduction by SRB, pH, and the production of ruminal H_2S. Recent studies evaluating dietary S level in cattle diets have primarily used acidic [e.g., H_2SO_4, various types of DGS, or $(NH_4)_2SO_4$] or a mixture of (e.g., DGS supplemented Na_2SO_4) dietary S sources. Felix et al. (2014) reported that at the same dietary S concentration, acidic dietary S sources (i.e., 60% DDGS or corn-based diet with H_2SO_4) increased ruminal H_2S gas concentrations and decreased DMI and ADG when compared to a neutral dietary S source (i.e., Na_2SO_4). These results suggest that when using neutral dietary S sources or adding neutral S sources to increase the S concentration of DGS based diets to test hypotheses on dietary S level, results may not generalize to acidic based diets. The reported results from studies evaluating dietary S level using Na_2SO_4 in ruminant diets on the productive and physiological responses of growing, finishing, and gestating animals are lacking. Accordingly, the objectives of the following research are to evaluate the effects of: 1) excess dietary S using supplemental Na_2SO_4 on steer performance, carcass characteristics, and beef quality after aging; 2) excess dietary S using supplemental Na_2SO_4 on beef shelf-life and fatty acid composition; 3) excess dietary S using supplemental Na_2SO_4 on growing and finishing steer mineral status, blood SHb concentrations, and CytOx activity; and 4) supplemental Cu to primiparous beef heifers consuming Cu deficient diets with or without excess dietary S using supplemental Na_2SO_4 pre- and postpartum on heifer and progeny productive and physiological responses.

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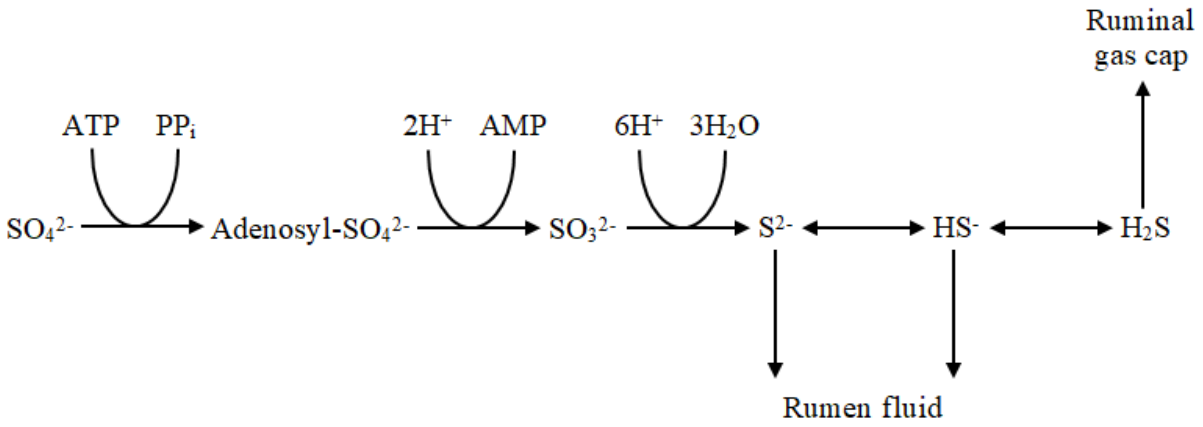

Figure 1. Bacterial sulfate (SO_4^{2-}) reduction (adapted from Russell, 2002). Sulfate reduction proceeds via the formation of adenosylsulfate (Adenosyl- SO_4^{2-}), sulfite (SO_3^{2-}), sulfide (S^{2-}), hydrosulfide (HS^-), and and finally hydrogen sulfide (H_2S). ATP = adenosine triphosphate; PP_i = pyrophosphate; H^+ = proton; AMP = adenosine monophosphate; and H_2O = water.

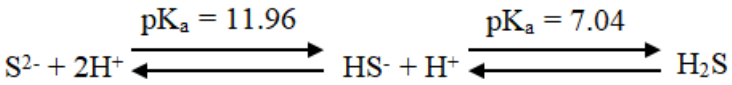

Figure 2. The chemical properties of hydrogen sulfide (H_2S; adapted from Beauchamp et al., 1984). Hydrogen sulfide is a weak acid and equilibrates with its anions hydrosulfide (HS^-) and sulfide (S^{2-}) in aqueous solution. Two acid dissociation constants (pK_a) are present: 1) as ruminal pH increases (greater than 7.04), the first dissociation occurs with ionization of a single proton (H^+) to yield hydrosulfide (HS^-); and 2) a second proton may dissociate from the resultant HS^- to yield S^{2-}. As these reactions are pH dependent, and ruminal pH is usually below 7.0, association towards H_2S formation is favored.

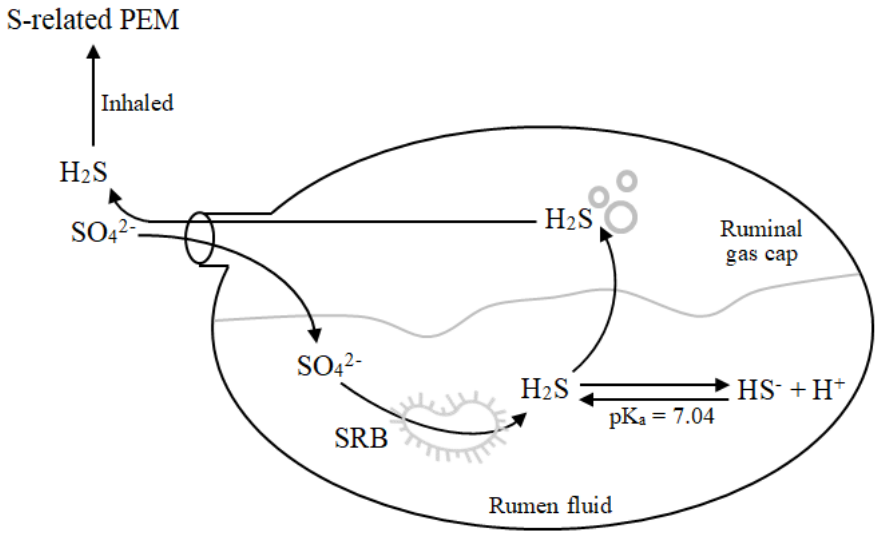

Figure 3. Ruminal metabolism of sulfate (SO_4^{2-}) by sulfur reducing bacteria (SRB) and the proposed pathway of S toxicity in ruminants (adapted from Drewnoski et al., 2014). Excess ruminally available S is reduced by dissimilatory SRB to hydrogen sulfide (H_2S) and excreted into the ruminal fluid. In a pH dependent manner, some of the H_2S will dissociate to hydrosulfide (HS^-) and remain in the rumen fluid, whereas the remaining H_2S will migrate to the ruminal gas cap. Based on the disassociation constant (pK_a), at pH = 7.0 in the rumen fluid 50% of H_2S will dissociate to HS^-, whereas at pH = 5.5 only 5% will dissociate to HS^-. Accumulated H_2S is eructated and subsequently inhaled. The inhaled cytotoxic H_2S enters the blood stream and can cause S-related polioencephalomalacia (PEM).

CHAPTER II

Excess dietary sulfur from sodium sulfate in beef steer growing-finishing diets: Effects on steer performance, carcass characteristics, and beef quality after aging

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ABSTRACT

The objective of this study was to determine the effects of excess dietary S using sodium sulfate (**Na_2SO_4**), a neutral dietary S source, on steer performance, carcass characteristics, and beef quality after aging. Twenty steers of predominantly Angus breeding were stratified by body weight (**BW**; 279 ± 13.2 kg), assigned randomly to 6, 2.4-ha paddocks (3 to 4 steers/paddock), and supplemented with either low S ground corn and soybean meal supplement that did not contain any byproduct feeds (0.31% total dietary S; **LS**) or LS supplement with an additional 0.25% S provided from Na_2SO_4 (0.58% total dietary S; **HS**) for 114 d. Steers were moved to feedlot (BW = 373 ± 0.2 kg), remained on prior dietary S treatments, and fed corn and soybean meal diets (0.19 and 0.42% total dietary S; LS and HS treatments, respectively) that did not contain any byproduct feeds with no use of growth-enhancing technologies for 123 d. Steers were harvested (BW = 564 ± 7.7 kg) in a commercial abattoir. Ribeye rolls (Institutional Meat Purchase Specifications #112) were vacuum-packaged aged for 14 d (4°C) and fabricated into 2.54-cm-thick longissimus muscle steaks. Consumer sensory panelists (n = 151) assessed cooked LM sensory attributes. Increasing the dietary S of growing diets with Na_2SO_4 did not affect ($P \geq 0.68$) performance responses in steers. However, differences were observed among treatments with regard to finishing performance. Increasing the S concentration of finishing diets with Na_2SO_4 decreased dry matter intake ($P < 0.001$) and average daily gain ($P = 0.07$); however, on

a carcass-adjusted basis, there were no ($P = 0.24$) treatment effects on average daily gain. No differences ($P > 0.15$) were identified among treatments with respect to carcass characteristics. Moreover, no differences ($P \geq 0.34$) were identified among treatments with respect to overall impression, tenderness, juiciness, beef flavor, and off-flavor. Shear force results were confirmed by consumer sensory panel results which indicated no difference ($P = 0.48$) and "just about right" consumer acceptability for tenderness. Consumer acceptability for flavor differed ($P = 0.07$) by treatment. Steaks from HS steers were rated as "too weak" by a greater percentage of consumers than steaks from LS steers (51.9 and 46.8%, respectively). These data suggest exposing growing-finishing cattle to greater S diets via supplemental dietary S from Na_2SO_4 has limited impact on steer performance, carcass characteristics, and beef quality after aging.

Key words: beef cattle, beef quality, carcass characteristics, performance, sodium sulfate

INTRODUCTION

Increased corn distillers grains (**DG**) production has led beef producers to use DG as a protein and energy supplement in cattle diets to maximize performance. The use of sulfuric acid (**H_2SO_4**) during ethanol production often results in an accumulation of excess S in DG. Accordingly, increased use of DG has led to considerable research evaluating the consequences of dietary S level in growing and finishing cattle diets. However, excess dietary S may not be the only factor limiting DG inclusion. The salient factor might be the reduction in dietary pH due to DG inherent acidity (Felix et al., 2012).

Studies evaluating dietary S level in cattle diets may use acidic {e.g., H_2SO_4, various types of DG, elemental S, or ammonium sulfate [**$(NH_4)_2SO_4$**]}, neutral [e.g., sodium sulfate (**Na_2SO_4**)], basic [e.g., calcium sulfate (**$CaSO_4$**)], or a mixture of dietary S sources. Felix et al. (2014) reported that at the same dietary S concentration, acidic dietary S sources [i.e., 60% corn dried distillers grains with solubles (**DDGS**) or corn-based diet with H_2SO_4] increased ruminal

H_2S gas concentrations and decreased dry matter intake (**DMI**) and average daily gain (**ADG**) when compared to a neutral dietary S source (i.e., Na_2SO_4). These results suggest that when using neutral dietary S sources or adding neutral S sources to increase the S concentration of DG based diets to test hypotheses on dietary S level, results may not generalize to acidic based diets.

Studies evaluating dietary S level using Na_2SO_4 in cattle diets are lacking, as confounding variables (e.g., DDGS plus Na_2SO_4) prohibit establishing a link between treatment and outcome. It was hypothesized cattle exposed to greater S diets via supplemental dietary S from Na_2SO_4 would experience fewer negative side effects on performance, carcass characteristics, and beef quality than high S diets that add acidic S sources to increase the S concentration. The objective of this study was to evaluate the effects of excess dietary S using supplemental Na_2SO_4 on steer performance, carcass characteristics, and beef quality after aging.

MATERIALS AND METHODS

Animal handling procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (protocol #13008) and followed guidelines recommended in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010). Procedures involving human subjects were approved by the University of Arkansas Institutional Review Board (IRB #13-06-755).

Animals and experimental design

Twenty steers [initial body weight (**BW**) = 279 ± 13.2 kg] of predominantly Angus breeding were obtained from the University of Arkansas Cow-Calf Unit (Savoy, AR). Steers were branded with a hot iron on the left hip, vaccinated with a multivalent clostridial bacterin-toxoid (Covexin 8; Merck Animal Health, Summit, NJ), and treated for internal (Synanthic; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO) and external parasites (Exit II; AgriPharm, Memphis, TN). Steers did not receive implants. Steers were stratified by initial BW

and assigned randomly to 1 of 6 paddocks (3 to 4 steers/paddock) for a 114-d growing phase. Paddocks were assigned randomly to 1 of 2 dietary treatments: 1) low S ground corn and soybean meal supplement (**LS**) or 2) LS supplement with an additional 0.25% S provided from Na_2SO_4 (**HS**; Table 1). Steers grazed mixed grass [0.31% S, 17.8% crude protein (**CP**), 26.8% acid detergent fiber (**ADF**), 57.1% neutral detergent fiber (**NDF**), and 10.4% ash, dry matter (**DM**) basis] paddocks (2.4-ha/paddocks) and were supplemented to meet NRC (2000) nutrient requirements for 0.15% S with a ground corn and soybean meal diet that did not contain any byproduct feeds (LS treatment). Remaining steers were offered an identical supplement to which an additional 0.25% S from Na_2SO_4 (Prince Agri Products, Inc., Quincy, IL) had been added in the total diet DM based on an estimated DMI of 6.59 kg/d (University of Arkansas, Division of Agriculture, Cooperative Extension Service, 2012). Due to the provision of S from Na_2SO_4, salt was incorporated into the LS treatment at an appropriate level to ensure that both treatments had similar Na levels. Daily amounts of supplement were weighed and provided at 0800 h to steers in their respective paddocks (0.5 m or more bunk space/steer). In the event forage became limiting, steers were provided access to bermudagrass hay (0.36% S, 18.8% CP, 33.0% ADF, 70.0% NDF, and 10.3% ash, DM basis) in quantities sufficient to ensure ad libitum access to forage. When the average BW of the steers reached 373 ± 0.2 kg, steers were stratified within dietary treatment by BW and assigned randomly to 16 dry-lot pens (1 to 2 steers/pen; 8 pens/dietary treatment). Steers remained on the same dietary treatment for a 123-d finishing phase. Steers on the LS treatment were offered a traditional corn and soybean meal finishing diet that did not contain any byproduct feeds or ionophore supplementation and met the NRC (2000) requirement of 0.15% S, whereas steers on the HS treatment steers were offered an identical diet except an additional 0.25% S was provided from Na_2SO_4 (Table 1). The feed management approach during

the finishing phase was designed to provide cattle with maximum daily intake using the slick-bunk feed management strategy. Feed bunks were visually evaluated each morning to determine the quantity of feed to offer each pen. Daily treatment amounts were weighed and provided at 0800 h to steers in their respective pens (0.5 m or more bunk space/steer) and free access to water. Steers were monitored daily for morbidity. Average daily gain was calculated using the total weight gained in each period divided by the length of each period. Feed efficiency was calculated using the total gain and the total DMI for each period of interest.

Sample collection and analytical procedures

Animals and diets. Steers were weighed on 2 consecutive days at the beginning and conclusion of each phase. Interim weights were collected every 28 d. During the growing phase, feed samples were collected daily and composited over 28 d periods, whereas hay samples were collected as bales were fed and composited over 28 d periods. Clipped forage samples (simulated grazed) were obtained every 28 d. Forage intake was not quantified. During the finishing phase, feed intake was quantified with samples collected daily and composited over 28 d periods. In the event of feed refusal, orts were collected, weighed and sampled for DM analysis. Feed refusals were subtracted from feed offered to calculate DMI. All forage and composited feed and hay samples were forced-aired dried (48 h at 50°C), ground to pass a 1-mm screen using a Wiley mill (Arthur H. Thomas Philadelphia, PA), analyzed in duplicate for DM (method 934.01; AOAC, 2000), and stored until analyzed. All dried samples were analyzed in duplicate for ash (method 924.05; AOAC, 1990), CP (method 990.03; AOAC, 1995; Elementar Americas, Inc., Mt. Laurel, NJ), NDF and ADF (Ankom Technology methods 5 and 6, respectively; ANKOM^{200} Fiber Analyzer; ANKOM Technology Corp., Fairport, NY), and digested using trace mineral-grade nitric acid with subsequent inductively coupled plasma atomic emission spectroscopy analysis

for complete minerals at a commercial laboratory (method 975.03; AOAC, 1988; Altheimer Laboratory, Fayetteville, AR). Instrument accuracy for mineral analyses was established using tomato standard (National Institute of Standards and Technology, Gaithersburg, MD). The pH of each diet was determined in duplicate by combining 20 g ground diet with 80 mL distilled water and mixing with a magnetic stir bar on a stir plate for 30 s then recording the pH with a calibrated portable pH meter (FG2 FiveGo; Mettler-Toledo, Columbus, OH).

Slaughter, steak fabrication, and pH. Feed was withheld overnight with free access to water, and all steers were transported 351 km for harvest in a commercial abattoir (Creekstone Farms, Arkansas City, KS). Individual identification was maintained with each carcass. Abattoir personnel recorded all carcass characteristics with the exception of yield and quality grades. Yield and quality grades were assigned by USDA line graders. Following a 48-h chilling period, the ribeye roll (Institutional Meat Purchase Specifications #112; USDA, 1996) was collected from both sides of the carcass during fabrication, vacuum-packaged, and transported on ice 372 km to the University of Arkansas Red Meat Abattoir (Fayetteville, AR). Ribeye rolls were aged 14 d (2°C) and fabricated into 7, 2.54-cm-thick longissimus muscle (**LM**) steaks. Starting at the caudal end of the rib section, 4 steaks were cut and used for the following analyses: Warner-Bratzler shear force (**WBSF**) and consumer sensory panel evaluation. Steaks were vacuum-packaged and stored at -20°C until analysis. Prior to steak fabrication, LM pH was measured in duplicate by inserting a calibrated hand-held pH meter with penetration probe (Testo 205; Testo SE & Co., Lenzkirch, Germany) directly into the meat.

Cooking loss and WBSF. Steaks were thawed for 24 h at 1°C. Steaks were cooked on electric griddles (National Presto Industries, Inc., Eau Claire, WI) and turned every 4 min until an internal temperature of 71°C was reached. Internal temperature was monitored with a digital

thermocouple thermometer (model KM28; Comark Instruments, Beaverton, OR). Cooked steaks were allowed to cool at room temperature for 1 h. Cooking loss was calculated by taking the initial weight of the steak prior to cooking. Cooked steaks were blotted dry on paper towels and reweighed. The difference between the cooked and initial weight was divided by the initial weight to calculate cooking loss percentage. Thereafter, a minimum of 7, 1.27-cm-diameter parallelepipeds were removed parallel with the muscle fiber orientation from each cooked steak. Shear force was measured using an Instron Universal Testing Machine (Instron Corp., Canton, MA) with a 50-kg compression load cell, crosshead speed of 200 mm/min, and Warner-Bratzler shear attachment. Shear force values of the cooked steaks were determined from sample parallelepiped means.

Consumer sensory panel. Consumer sensory panelists (n = 151) assessed cooked LM sensory attributes. Frozen steaks were shipped frozen to the University of Arkansas Sensory Service Center (Fayetteville, AR) and thawed in a 4°C refrigerator for 24 h before sensory panels. Steaks were cooked to an internal temperature of 71°C on electric griddles (National Presto Industries, Inc.), removed, then rested uncovered for 5 min. Internal temperature was monitored with a digital thermocouple thermometer (Comark Instruments). Steaks were cut into 1.27 × 1.27 × 2.54-cm samples, covered, and placed in a warming oven (60°C) until panelists were served. Samples were evaluated in triplicate over 16 panels with 2 samples/panel (1, LS sample and 1, HS sample/panel). Panelists were seated randomly at temperature controlled bread-box hood sensory analysis booths containing red filtered lighting to reduce visible effects due to cooking method, degree of doneness, or muscle effect. Sample serving sequence was randomized and counterbalanced based on Williams (1949) sequential design as generated by Compusense at-hand (Compusense Inc., Guelph, ON, Canada) sensory evaluation software. The

design provided a balance for treatment frequency, order, and carryover effects. Panelists rated each steak sample using 9-point hedonic scales for overall tenderness, juiciness, and beef flavor (1 = dislike extremely to 9 = like extremely), and off-flavor intensity (1 = no off-flavor to 9 = extremely strong off-flavor). A just-about-right (**JAR**) scale (5-point scale; 3 = just about right) characterized the influence of flavor and tenderness on consumer acceptability. Panelists were provided spring drinking water and saltless saltine crackers to cleanse their palates and to minimize sensory fatigue between samples.

Statistical analysis

Weight, carcass characteristics, and beef quality data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Inst., Inc., Cary, NC) by means of a first-order autoregressive covariance structure. Denominator degrees of freedom were estimated using the Kenward-Rogers option. Adherence of the data to the assumptions of the statistical test was established. Weight data were analyzed as repeated measures. Fixed effects included in the model were dietary treatment and day, as well as the 2-way interaction, whereas paddock (experimental unit) was included as a random effect. Day was included as a repeated effect. Carcass characteristics (HCW, dressing percentage, USDA yield grade, LM area, 12th-rib fat thickness, pH, drip loss, cooking loss, and WBSF) were analyzed as randomized designs. Dietary treatment was the fixed effect and pen (experimental unit) was included as the random effect in the models. Consumer sensory data were analyzed as a randomized design. Dietary treatment was the fixed effect. Panel was included in the model as a covariate when significant. Least squares means were partitioned at the 10% level of significance by way of the probability of differences option. Effect of treatment on USDA quality grade was analyzed using the GLIMMIX procedure of SAS. Ordinal data were tested using a multinomial distribution,

cumulative logit link function, and variance components covariance structure. Pen (experimental unit) was included in the model as a random effect. Statistical significance was declared at $P \leq 0.10$.

RESULTS AND DISCUSSION

The objective of this study was to evaluate the effects of excess dietary S using supplemental Na_2SO_4, a neutral dietary S source, on steer performance, carcass characteristics, and beef quality after aging. It was hypothesized that cattle exposed to greater S diets via supplemental dietary S from Na_2SO_4 would experience fewer negative side effects of high S diets on performance, carcass characteristics, and beef quality. These data suggest exposing growing-finishing cattle to greater S diets via supplemental dietary S from Na_2SO_4 has limited impact on steer performance, carcass characteristics, and beef quality after aging. However, when interpreting the results of this study, there are a couple factors that should be considered. Greater steer numbers would increase the power for detection of performance, carcass characteristics, and beef quality differences. In light of the small sample size per treatment group, the power to detect significant differences was limited and a factor in the choice to use a type I error rate of 0.10. Moreover, the lack of studies that used Na_2SO_4 for comparison prohibited establishing a substantiated relationship between treatment and outcome.

Diet and water analyses

Ground corn and soybean meal supplements fed during the growing phase contained 0.30 and 1.31% S for LS and HS treatments, respectively. Forage was analyzed to contain 0.31% S. Growing phase total dietary S concentrations of 0.31 and 0.58% for LS and HS treatments, respectively, were calculated by multiplying estimated forage and supplement DMI by their respective S concentrations. The DM contribution of S from hay was excluded from the calculation, as forage was seldom limiting. The total dietary S concentration for the HS treatment

during the growing phase met the condition of "excess dietary S" as it exceeded the 0.50% of dietary DM suggested by NRC (2005) as the maximum tolerable concentration for high roughage diets. The theoretical difference in S concentration between growing diets was 0.25%, whereas the analyzed difference was 0.27%. Ground corn and soybean meal diets fed during the finishing phase were analyzed to contain 0.19 and 0.42% S for the LS and HS treatments, respectively. The total dietary S concentration for the HS treatment during the finishing phase met the condition of "excess dietary S" as it exceeded the 0.30% of dietary DM suggested by NRC (2005) as the maximum tolerable concentration for high concentrate diets. The theoretical difference in S concentration between finishing diets was 0.25%, whereas the analyzed difference was 0.23%.

A composite water sample from a later study conducted in the same location as the current study was subjected to complete mineral analysis by a commercial laboratory (methods 200.7 and 300.0; EPA, 1993; Arkansas Water Resources Center, Fayetteville, AR). Water sulfate (**SO_4^{2-}**) concentration was reported as 1.48 mg SO_4^{2-}/L. Sulfate is approximately 33.4% elemental S. Accordingly, if water consumption averaged 34 L/steer (NRC, 2000), the DM contribution of S from water in addition to the S provided in each dietary treatment was insignificant in the current experiment. Similarly, the DM contribution of the other analyzed mineral concentrations from water was insignificant.

Performance

Excess dietary S can negatively affect cattle, including decreased performance, polioencephalomalacia (**PEM**), and in some cases death (Gould, 1998). No steers were treated for or developed clinical PEM symptoms during the study.

Growing phase. Under the conditions of the current study, HS steers were able to tolerate greater S intake than the 0.50% of dietary DM suggested by NRC (2005) as the maximum

tolerable concentration for high roughage diets when supplementing Na_2SO_4 as a dietary S source. Increasing the S concentration of growing diets from 0.31 to 0.58% S with Na_2SO_4 did not affect ($P \geq 0.68$) the performance responses in steers. Consistent with these findings, research by Digesti and Weeth (1976) reported that increased dietary S, supplied in the drinking water, did not affect the performance of growing heifers. In their study, supplementing drinking water with Na_2SO_4 [110, 1,250, and 2,500 mg SO_4^{2-}/L; 0.20, 36, and 0.57% total (water plus feed) dietary S, respectively] to increase the total dietary S intake did not affect DMI or ADG. Conversely, Cammack et al. (2010) and Weeth and Capps (1972) reported increasing the total dietary S intake of growing cattle with supplemental Na_2SO_4 impacted performance. Cammack et al. (2010) administered drinking water supplemented with Na_2SO_4 [566 and 3,651 mg SO_4^{2-}/L; 0.28 and 0.79% total (water plus feed) dietary S, respectively] to forage-fed steers. Increasing total dietary S from 0.28 to 0.79% decreased intake in the first 53 d of the study; however, DMI was not affected by dietary S in the final 23 d of the study. Similarly, Weeth and Capps (1972) observed increasing the total dietary S from 0.41 to 0.63% decreased the intake of growing heifers when supplementing drinking water with Na_2SO_4 [110, 1,462, and 2,814 mg SO_4^{2-}/L; 0.20, 0.41, and 0.63% total (water plus feed) dietary S, respectively]. Differences in growing cattle performance results when evaluating dietary S level using Na_2SO_4 may be attributed to differences in the ranges of dietary S concentrations evaluated and study duration compared to the current study. The greatest dietary S concentration in Weeth and Capps (1972) and Cammack et al. (2010) were 0.63 and 0.79%, respectively. Research indicates that sulfate reducing bacteria (**SRB**) in the rumen require time to adapt to dietary S, especially when transitioning cattle to diets that exceed the maximum tolerable level of S (Drewnoski et al., 2012; Felix et al., 2014). Results from Cammack et al. (2010) suggest an adaptation of SRB in the rumen to dietary S.

Increasing dietary S decreased intake in the first 53 d of the study, whereas intake was not affected by dietary S in the final 23 d of the study. However, when combining chronic exposure to increased dietary S with a 30-d trial, Weeth and Capps (1972) may not have allowed sufficient time for the adaptation of SRB to the increased ruminal SO_4^{2-} concentration.

Reported effects of increased dietary S from acidic dietary S sources on performance responses in growing cattle are variable. Consistent with the current study, increasing the S concentration in grasses from 0.22 to 0.51% with $(NH_4)_2SO_4$ fertilizer (Arthington et al., 2002) or from 0.12 to 0.46% in a corn silage-based growing diet with $(NH_4)_2SO_4$ (Spears et al., 2011) did not affect DMI in growing cattle. Conversely, Buttrey et al. (2012) found acidic dietary S source supplementation during the growing phase altered performance. Steers supplemented DDGS at 0.5% of BW (0.40% total dietary S) during the growing phase gained more and had greater final BW than steers provided no supplement or supplemental dry rolled corn. Acidic ruminal pH can reduce the activity of cellulolytic and proteolytic bacteria to decrease dietary fiber and protein fermentation. Because $(NH_4)_2SO_4$ and DDGS are inherently acidic, it is conceivable that the S sources would decrease animal performance owing to reduced dietary fiber and protein digestion. However, the high-fiber diets likely promoted chewing and saliva production, to buffer ruminal pH and mitigate differences when comparing Na_2SO_4 with $(NH_4)_2SO_4$ and DDGS as dietary S sources in growing cattle diets. Positive responses may be attributed to the buffering capacity of the high-fiber diets combined with the high energy value of DDGS.

Finishing phase. Under the conditions of the current study, HS steers were able to tolerate greater S intake than the 0.30% of dietary DM suggested by NRC (2005) as the maximum tolerable concentration for high concentrate diets when supplementing Na_2SO_4 as a

dietary S source. Increasing the dietary S of finishing diets from 0.19 to 0.42% S with Na_2SO_4 decreased DMI ($P < 0.001$) and ADG ($P = 0.07$), but gain:feed was not affected ($P = 0.60$; Table 2). These findings do not support the hypothesis that cattle exposed to greater S diets via supplemental dietary S from Na_2SO_4 would experience fewer side effects of high S diets on performance. Sodium sulfate provides both a bitter and saline taste. Accordingly, the Na_2SO_4 in the current study may have been mixed with an insufficient amount of other feedstuffs (e.g., molasses, grain, etc.) to overcome any palatability problems to result in decreased ADG and DMI. However, on a carcass-adjusted basis, there were no ($P = 0.24$) treatment effects on ADG. Treatments did not ($P \geq 0.15$) influence calculated dietary energy. The difference in finishing performance (1.38 and 1.20 kg/d; LS and HS steers, respectively) when compared to the industry average (1.57 kg/d; Zinn et al., 2008) was attributed to not using growth-promoting technologies or ionophore supplementation. Contrary to the current study, increasing dietary S to 0.12, 0.30, and 0.37% in a ground corn-based finishing diet with Na_2SO_4 increased ADG and improved feed efficiency in lambs fed both 0.30 and 0.37% S (Felix et al., 2014). The authors suggested that the improvement was unexpected and likely unique to the study as it occurred when lambs were fed dietary S in excess of NRC (2007) recommendations. With high grain consumption, the concentration of dissociated organic acids can become quite high, resulting in rumen pH dropping below 6. The ionophores monensin and lasalocid shift the ruminal bacteria population and metabolism to increase the production of propionic acid while decreasing the production of acetic and lactic acids. As propionic acid is a weaker acid than acetic and lactic acids (pKa = 4.9, 4.8, and 3.8, respectively), it may contribute to an increase in ruminal pH. Felix et al. (2014) fed lambs 19.1 mg lasalocid/kg of the diet DM. Therefore, it is unlikely that the improvement observed by Felix et al. (2014) was owing to an artifact of their study. The supplementation of

Na_2SO_4 and potentially greater ruminal pH from lasalocid likely caused an additive response to improve performance.

A reduction in feedlot performance is primarily observed with increased inclusion of acidic dietary S sources. Bolsen et al. (1973) and Spears et al. (2011) observed a decrease in DMI when steers were fed $(NH_4)_2SO_4$ as a supplemental source of dietary S in beef cattle finishing diets (0.41% and 0.47% S, respectively). Felix et al. (2012) observed DMI linearly decrease with increasing levels of DDGS (0, 20, 40, or 60% DM; 0.11, 0.20, 0.31, or 0.43% S, respectively) replacing rolled corn. When cattle were fed a 60% DDGS diet (0.43% S), ADG and DMI decreased 16 and 13%, respectively, when compared to a 0% DDGS corn-based control diet (0.12% S; Felix et al., 2014). Sarturi et al. (2013) reported steers fed increasing levels of a 0.82% S DDGS (20, 30, or 40% DM; 0.26, 0.33, or 0.40% S, respectively) diet experienced a linear increase in DMI when compared to steers fed a 0% DDGS corn-based control (0.13% S) diet. However, steers fed increasing levels of a 1.16% S DDGS (20, 30, or 40% DM; 0.33, 0.43, or 0.54% S, respectively) diet experienced a quadratic decrease in DMI when compared to the same 0% DDGS corn-based control (0.13% S) diet.

Ruminal hydrogen sulfide (**H_2S**) concentration is related to PEM when excess dietary S is fed to ruminants (Gould, 1998). However, before an animal exhibits clinical symptoms of PEM, performance can decrease (Thompson et al., 1972; Bolsen et al., 1973). Gould (1998) suggested that ruminal pH and H_2S concentration may be related, as low pH favors the reduction of S to H_2S in the rumen. With a change in ruminal pH from 6.8 to 5.2, the percent H_2S in the rumen gas cap was observed to increase from 46.8 to 97.2% (Gould, 1998). Gould et al. (1997) suggested that 2,000 mg H_2S/L is the concentration at which cattle are at risk of developing PEM. Increasing dietary S to 0.12, 0.30, and 0.37% in a ground corn-based finishing diet with Na_2SO_4

increased ADG and improved feed efficiency with a concomitant increase in ruminal H_2S concentrations in lambs fed 0.30 and 0.37% S (Felix et al., 2014). However, ruminal H_2S concentrations peaked below the suggested 2,000 mg H_2S/L threshold at 1,131 and 1,495 mg H_2S/L for the lambs fed 0.30, and 0.37% S, respectively. Pogge et al. (2016) fed steers differing inclusions of DDGS (20, 40, or 60% DM; 0.28, 0.41, or 0.56% S, respectively) replacing dry-rolled corn. With increasing DDGS inclusion, final BW linearly decreased with a concomitant increase in ruminal H_2S concentrations (1,054, 3,155, and 4,298 mg H_2S/L; 0, 20, or 40% DDGS, respectively). In the previously mentioned study by Sarturi et al. (2013), steers fed DDGS containing 1.16% S had less DMI than steers fed DDGS with 0.82% S, and the former tended to have greater ruminal H_2S concentrations than the latter. Crane et al. (2017) observed ruminal H_2S concentrations linearly increase with increasing DDGS inclusion (0, 15, or 30% DM; 0.2, 0.3, or 0.4% S, respectively) replacing corn. There was a tendency for decreased ruminal pH with increased DDGS inclusion (4.96, 4.90, and 4.85; 0, 15, or 30% DDGS, respectively). It is conceivable the observed differences in finishing performance when supplementing various dietary S sources may reflect ruminal pH as ruminal H_2S concentration may increase when more hydrogen ions are available (i.e., acidic dietary S source) to form H_2S. Therefore, finishing diets containing excess dietary S from neutral S sources may be less detrimental to cattle performance when compared to diets containing excess dietary S from acidic S sources. As ruminal pH is influenced by readily fermentable substrates, it is important to note these results may be confounded by differing inclusions of corn. Moreover, differences in finishing performance associated with differing DDGS inclusion may be attributed not only to ruminal pH, but also to dietary fat not being held constant across diets.

Carcass characteristics

Exposing cattle to excess dietary S via supplemental dietary S from Na_2SO_4 did not affect ($P > 0.15$; Table 3) carcass characteristics. The lack of differences in 12th-rib fat thickness due to dietary treatment was expected as steers were finished to a common thickness for harvest. Reported effects of increased dietary S from acidic dietary S sources on carcass characteristics have varied among studies. Spears et al. (2011) fed steers differing inclusions of $(NH_4)_2SO_4$ (0.13, 0.31, and 0.46% S) in a corn-based finishing diet. Carcass characteristics did not differ among steers fed diets containing 0.13 and 0.31% S, whereas steers fed diets containing 0.46% S had decreased hot carcass weight (**HCW**), LM area, quality, and yield grade compared to steers fed 0.13% S. Increasing DDGS inclusion (20, 40, or 60% DM; 0.28, 0.41, or 0.56% S, respectively) linearly decreased HCW and increased dressing percentage, whereas marbling scores showed a quadratic response with optimum inclusion at 40% (Pogge et al., 2016). Luebbe et al. (2012) reported HCW, 12th-rib fat thickness, marbling score, and calculated yield grade linearly decreased with increasing wet corn distillers grains with solubles (**WDGS**) inclusion (15, 30, 45, or 60% DM; 0.22, 0.29, 0.36, or 0.44% S, respectively). Buckner et al. (2008) fed increasing levels of DDGS (0, 10, 20, 30, or 40% DM; 0.15, 0.24, 0.33, 0.41, or 0.55% S, respectively) replacing dry-rolled corn and observed a quadratic response for HCW with optimum inclusion at 20%.

Differences observed between results reported in the present study and those utilizing acidic dietary S sources could be attributed to starch availability and differences in extent of ruminal and post-ruminal starch digestion, and competition between hydrogen (**H_2**) utilizing bacteria in the rumen. In the current study, corn was the predominant energy source in the finishing diets with energy primarily due to starch. Starch is rapidly fermented by ruminal

microorganisms into propionate. If not digested in the rumen, starch reaches the small intestine and is digested by pancreatic amylase directly into glucose. Glucose is transported across the enterocyte brush-border and absorbed via the Na-dependent glucose co-transporter-1 (Ladeira et al., 2016). Glucose is the primary substrate for intramuscular adipose tissue development (Smith and Crouse, 1984). It is possible that carcass characteristics did not differ in the current study as the diets provided similar amounts of intestinal starch and Na for intestinal glucose absorption. Conversely, starch availability and differences in extent of ruminal and post-ruminal starch digestion have been reported to influence how DG affect carcass characteristics. Corrigan et al. (2009) found increased corn processing (dry-rolled corn, high-moisture corn, or steam-flaked corn), when combined with increasing levels of WDGS (0, 15, 27.5, or 40% DM), affected carcass characteristics more severely at greater inclusion rates.

Ruminal carbohydrate-fermenting bacteria require continuous removal of H_2 for sustained organics matter digestion and volatile fatty acid production (Sharp et al., 1998). On growing diets, methanogenic bacteria are primarily responsible for removing H_2 produced during microbial fermentation via methanogenesis. Methanogens are sensitive to ruminal pH with methanogenesis essentially halting at pH < 6.0 (Van Kessel and Russell, 1996). On finishing diets, the drop in ruminal pH inhibits methanogens while carbohydrate-fermenting bacteria utilize propionate producing bacteria as an alternative mechanism for H_2 removal (Ellis et al., 2008). The addition of Na_2SO_4 to *in vitro* cultures (0.17 to 0.42% S) using a steam-flaked corn substrate increased the molar proportion of propionate with a concomitant decrease in the acetate:proprionate (**A:P**) ratio (Quinn et al., 2009). It is possible, however, that on a high S diet the number of SRB can readily surpass propionate producing bacteria to compete for available H_2 (Ellis et al., 2008). Propionate may be a gluconeogenic precursor for the synthesis of

intramuscular fatty acids (Ladeira et al., 2016). Therefore, the adaptation of SRB to decreased ruminal pH and increased S concentrations from an acidic dietary S source could potentially decrease molar proportions of propionate to influence carcass characteristics to a greater extent than a neutral dietary S source. Sarturi et al. (2013) reported steers fed a 1.16% S DDGS (40% DM; 0.54% S) diet had lower molar proportions of propionate and tended to have a greater A:P ratio compared to steers fed 0.82% S DDGS (40% DM; 0.40% S). When feeding steers identical diets in another trial, steers fed the 1.16% S DDGS diet experienced lower HCW and 12th-rib fat thickness when compared with steers fed the 0.82% S DDGS diet. Accordingly, the lower molar proportions of propionate resulting from the 1.16% S DDGS diet were consistent with decreased carcass characteristics. Luebbe et al. (2012) reported molar proportions of acetate increased linearly whereas molar proportions of propionate decreased linearly with increasing WDGS inclusion (15, 30, 45, or 60% DM; 0.22, 0.29, 0.36, or 0.44% S, respectively). When feeding steers identical diets in another trial, the concomitant relationship between increasing WDGS inclusion and a linear decrease in molar proportions of propionate was consistent with a linear decrease in HCW, 12th-rib fat thickness, marbling score, and calculated yield grade. Schoonmaker et al. (2010) found the molar proportions of volatile fatty acids were not different among steers fed differing amounts of wet distillers grains (0, 20, or 40% DM; 0.16, 0.23, or 0.33% S, respectively). However, increasing wet distillers grains inclusion linearly increased LM area and decreased fat thickness, marbling score, percentage of carcasses grading Choice or greater, and yield grade. Differences between results reported by Schoonmaker et al. (2010) and those by Sarturi et al. (2013) and Luebbe et al. (2012) could be attributed to S concentrations evaluated. Dietary S concentrations in Schoonmaker et al. (2010) may have been insufficient to change ruminal microbial population dynamics.

Beef quality

Cooking loss and WBSF. Feeding steers excess dietary S via supplemental Na_2SO_4 did not ($P \geq 0.18$; Table 4) influence cooking loss or WBSF values. Chao et al. (2017) also failed to detect differences in WBSF values of strip loin steaks aged 14 d from steers fed a 50% WDGS diet (0.41% S) when compared to strip loin steaks aged 14 d from steers fed a 0% corn-based control diet (0.09% S). Similar results were documented by Domenech-Pérez et al. (2017). Feeding steers varying levels of WDGS (0, 35, 50, or 65% DM; 0.09, 0.31, 0.41, or 0.51% S, respectively) did not influence the WBSF values of strip loin steaks aged 7 and 21 d. At 14 d of aging, WBSF values of the current study would be considered satisfactory for tenderness by consumers (<3.0 kg at d 14 for 100% consumer tenderness acceptability; Miller et al., 2001). Along with diet, muscle pH can influence meat tenderness. In the current study, feeding varying levels of dietary S did not ($P = 0.37$) influence muscle pH. The difference in WBSF values (2.26 and 2.42 kg; LS and HS steers, respectively) when compared to the industry average (3.45 kg; Reuter et al., 2002) for rib/chuck rolls was attributed to steers of predominantly Angus breeding (Binder et al., 2002; Wheeler et al., 2010) and not utilizing growth-promoting technologies (Garmyn and Miller, 2014).

Consumer sensory panel. Demographic analysis of panelist ($n = 151$) responses indicated all consumed beef. Consumers were predominantly female (68.9%). Yearly individual income and age were diverse. Because the sample represented consumers from diverse age levels, income levels, and included consumers categorized as "beef-eaters," the demographic profile was deemed an acceptable sample to assess if excess dietary S using supplemental Na_2SO_4 in growing and finishing cattle diets influenced consumer perception of beef palatability attributes. No differences ($P \geq 0.34$; Table 5) were identified among treatments with respect to overall impression, tenderness, juiciness, beef flavor, and off-flavor. Buttrey et al. (2013) also

failed to detect differences in sensory attributes of LM steaks aged 14 d from steers fed a 35% WDGS diet (0.34% S) when compared to LM steaks aged 14 d from steers fed a 0% corn-based control diet (0.12% S). The WBSF results of the current study were confirmed by the results of the consumer sensory panel which indicated no difference ($P = 0.48$) and "just about right" consumer acceptability for tenderness. Interestingly, despite similar consumer acceptability for tenderness, the percentage of consumers dissatisfied with tenderness was surprisingly high. Steaks from LS steers were rated as "too tough" by a numerically greater percentage of consumers than steaks from HS steers (36.4 and 31.6%, respectively). The means for beef flavor are reported in Table 5. The greater the number, the more the consumer liked the beef flavor. As results from the consumer panel indicated no difference ($P = 0.39$) in beef flavor, which averaged 6.30 ± 0.06 (9-point hedonic scale), it was anticipated that consumers would report that treatment did not influence flavor acceptability. However, consumer acceptability for flavor differed ($P = 0.07$) by treatment. Steaks from HS steers were rated as "too weak" by a greater percentage of consumers than steaks from LS steers (51.9 and 46.8%, respectively). Consumers often generalize and misevaluate sensory attributes because of a less favorable evaluation of another attribute. This is similar in principle to the halo effect (Roeber et al., 2000), and occurs when multiple attributes of a single sample are evaluated at the same time and how they are evaluated is influenced by the others. For example, if a steak is being evaluated on its overall impression, tenderness, juiciness, beef flavor, and also the sample's off-flavor intensity, then a sample which was favored less may receive less favorable ratings for each of the attributes individually as well. Accordingly, as the percentage of consumers dissatisfied with tenderness was surprisingly high, consumers were apt to be dissatisfied with flavor too.

In conclusion, these data suggest exposing growing-finishing cattle to greater S diets via supplemental dietary S from Na_2SO_4 has limited impact on steer performance, carcass characteristics, and beef quality after aging. Under the conditions of the current study, HS steers were able to tolerate greater S intake than the 0.50% of dietary DM suggested by NRC (2005) as the maximum tolerable concentration for high roughage diets when supplementing Na_2SO_4 as a dietary S source. Moreover, HS steers were able to tolerate greater S intake than the 0.30% of dietary DM suggested by NRC (2005) as the maximum tolerable concentration for high concentrate diets when supplementing Na_2SO_4 as a dietary S source.

ACKNOWLEDGMENTS

This research was supported by the Arkansas Beef Council. The authors thank J. Reynolds, J. Hornsby (retired), D. Bignar, D. Galloway, and T. Johnson (University of Arkansas, Department of Animal Science, Fayetteville, AR) and A. Ryan (Renaissance Nutrition, Inc., Roaring Spring, PA) for assistance with animal care, meat fabrication, and data collection.

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Table 1. Ingredient and analyzed composition of corn and soybean meal diets fed to steers.

	Treatments			
	Growing phase[1,2]		Finishing phase[3]	
Item	LS	HS	LS	HS
Ingredient, % as-fed basis				
Corn grain, cracked	81.2	80.4	76.3	76.1
Bermudagrass hay	--	--	7.0	7.0
Soybean meal	12.6	12.6	6.0	6.0
Cottonseed hulls	--	--	5.0	5.0
Molasses, cane	1.8	1.8	3.0	3.0
Calcium carbonate	0.45	0.45	1.0	1.0
Salt	3.30	0.00	0.85	0.00
Sodium sulfate, anhydrous[4]	0.00	4.10	0.00	1.05
Urea	--	--	0.80	0.80
Trace mineral premix[5]	0.10	0.10	0.025	0.025
Vitamin A, D, and E[6]	0.10	0.10	0.05	0.05
Rumensin premix[7]	0.40	0.40	--	--
Analyzed composition, DM basis[8]				
CP, %	14.9	14.5	14.3	15.1
ADF, %	2.1	2.0	6.4	6.6
NDF, %	16.5	17.2	23.7	27.7
Ca, %	0.48	0.38	0.68	0.66
P, %	0.43	0.40	0.34	0.36

Table 1 (Cont.)

	Treatments			
	Growing phase[1,2]		Finishing phase[3]	
Item	LS	HS	LS	HS
S, %	0.30	1.31	0.19	0.42
Na, %	1.96	1.73	0.43	0.37
Cu, mg/kg	73	60	24	18
Zn, mg/kg	270	239	108	85
Diet pH	5.18	5.24	5.36	5.47

[1]Fed at a rate of 1.8 $kg \cdot d^{-1} \cdot hd^{-1}$ to cattle grazing mixed grass pasture and also offered ad libitum access to bermudagrass hay.
[2]LS = low S, 0.31% total dietary S and HS = high S, 0.58% total dietary S.
[3]LS = low S, 0.19% total dietary S and HS = high S, 0.42% total dietary S.
[4]Prince Agri Products, Inc., Quincy, IL.
[5]Trace mineral premix composition (mg/kg): 500 Co; 40,000 Cu; 2,000 I; 10,000 Fe; 80,000 Mn; 600 Se; and 120,000 Zn (Nutrablend, Neosho, MO).
[6]Vitamin premix composition (IU/kg): 1,816,000 vitamin A; 36,320 vitamin D; and 227 vitamin E.
[7]Rumensin 80 (Elanco, Indianapolis, IN) supplied 22 mg of monensin/kg of diet DM.
[8]CP = crude protein; ADF = acid detergent fiber; and NDF = neutral detergent fiber.

Table 2. Least square means for the effects of excess dietary S from supplemental sodium sulfate on steer performance and dietary energy content.

	Treatment[1]			
Item[2]	LS	HS	SEM	*P*-value
Growing phase				
Paddocks (steers)	3 (10)	3 (10)		
Initial BW, kg	281	277	18.7	0.90
Final BW, kg	401	401	18.7	0.98
ADG, kg/d	1.07	1.11	0.06	0.68
Finishing phase				
Pens (steers)	8 (10)	8 (10)		
Initial BW, kg	401	401	18.7	0.98
Final live BW, kg	575	554	10.9	0.18
Final shrunk BW,[3] kg	549	530	11.9	0.30
ADG, kg/d	1.38	1.20	0.06	0.07
DMI, kg/d	11.96	10.62	0.20	<0.001
G:F	0.12	0.11	0.006	0.60
Dietary energy[4]				
ME, Mcal/kg	2.51	2.58	0.03	0.15
NEm, Mcal/kg	1.62	1.68	0.03	0.15
NEg, Mcal/kg	1.02	1.06	0.03	0.16

Table 2 (Cont.)

Item[2]	Treatment[1]		SEM	*P*-value
	LS	HS		
Total system BW gain,[5] kg	289	276	10.2	0.37
Carcass-adjusted performance				
Final BW,[6] kg	552	538	12.7	0.48
ADG, kg/d	1.19	0.99	0.25	0.24
G:F	0.11	0.10	0.006	0.70
Total system BW gain, kg	270	261	10.1	0.57

[1]LS = low S and HS = high S.
[2]BW = body weight; ADG = average daily gain; DMI = dry matter intake; G:F = gain to feed ratio; ME = metabolizable energy; NEm = net energy for maintenance; and NEg = net energy for gain.
[3]Final shrunk BW = final live (unshrunk) BW × 0.96 (NRC, 1996).
[4]Dietary NE calculated from performance data using methodology by Vasconcelos and Galyean (2008).
[5]Total system BW gain calculated from sum of BW gained during growing phase and BW gained during finishing phase.
[6]Carcass-adjusted final BW calculated from hot carcass weight divided by average dressing percent of all treatments.

Table 3. Least square means for the effects of excess dietary S from supplemental sodium sulfate on beef carcass characteristics.

Item	Treatment[1]		SEM	*P*-value
	LS	HS		
Hot carcass weight, kg	361	350	7.30	0.32
Dressing percent	64.4	65.3	0.41	0.15
USDA yield grade[2]	2.96	3.18	0.22	0.50
USDA quality grade[3]			1.01	0.40
Choice	7	5		
Prime	3	5		
Longissimus muscle area, cm^2	83.47	79.83	2.01	0.23
12th-rib fat thickness, mm	11.7	13.2	0.15	0.50

[1]LS = low S and HS = high S.
[2]2 = high standard and 3 = low select.
[3]Frequency within treatment.

Table 4. Least square means for the effects of excess dietary S from supplemental sodium sulfate on muscle pH, drip loss, cooking loss, and Warner-Bratzler shear force.

	Treatment[1]			
Item	LS	HS	SEM	*P*-value
Muscle pH	5.51	5.53	0.02	0.37
Drip loss, %	5.08	5.06	0.23	0.97
Cooking loss, %	24.81	25.78	0.60	0.26
Warner-Bratzler shear force, kg	2.26	2.42	0.08	0.18

[1]LS = low S and HS = high S.

Table 5. Least square means for the effects of excess dietary S from supplemental sodium sulfate on consumer sensory panel (n = 151) steak longissimus muscle ratings.

	Treatment[1]			
Characteristic[2]	LS	HS	SEM	*P*-value
Overall impression	6.43	6.53	0.09	0.34
Tenderness	6.49	6.59	0.08	0.37
Juiciness	6.10	6.13	0.08	0.78
Beef flavor	6.23	6.33	0.08	0.39
Off-flavor intensity	3.70	3.68	0.10	0.86
Tenderness JAR[3]	2.64	2.66	0.03	0.48
Flavor JAR[4]	2.52	2.43	0.06	0.07

[1]LS = low S and HS = high S.
[2]Overall impression, tenderness, juiciness, and beef flavor were evaluated on a 9-point hedonic scale, where 1 = dislike extremely to 9 = like extremely. Off-flavor was evaluated on a 9-point hedonic scale, where 1 = no off-flavor to 9 = extremely strong off-flavor.
[3]JAR = just-about-right; 5-point JAR scale, where 1 = much too tough to 5 = much too tender.
[4]Five-point JAR scale, where 1 = much too weak to 5 = much too strong.

CHAPTER III

Excess dietary sulfur from sodium sulfate in beef steer growing-finishing diets: Effects on beef shelf-life and fatty acid composition

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ABSTRACT

The objective of this study was to determine the effects of excess dietary S using sodium sulfate (**Na_2SO_4**) on beef shelf-life and fatty acid (**FA**) composition. Twenty steers of predominantly Angus breeding were stratified by body weight (**BW**; 279 ± 13.2 kg), assigned randomly to 6, 2.4-ha paddocks (3 to 4 steers/paddock), and supplemented with either low S ground corn and soybean meal supplement that did not contain any byproduct feeds (0.31% total dietary S; **LS**) or LS supplement with an additional 0.25% S provided from Na_2SO_4 (0.58% total dietary S; **HS**) for 114 d. Steers were moved to feedlot (BW = 373 ± 0.2 kg), remained on prior dietary S treatments, and were fed corn and soybean meal diets (0.19 and 0.42% total dietary S; LS and HS treatments, respectively) that did not contain any byproduct feeds with no use of growth-enhancing technologies for 123 d. Steers were harvested (BW = 564 ± 7.7 kg) in a commercial abattoir. Ribeye rolls (Institutional Meat Purchase Specifications #112) were vacuum-packaged aged for 14 d (4°C) and fabricated into 2.54-cm-thick longissimus muscle (**LM**) steaks. Steaks were placed under retail display (**RD**) conditions for 7 d. Feeding steers excess dietary S via supplemental Na_2SO_4 did not influence LM proximate composition ($P \geq 0.44$) or thiobarbituric acid reactive substances ($P > 0.42$). A treatment × day interaction ($P < 0.01$) was detected for worst-point color when evaluated by a trained visual panel. At d 4, steaks from steers fed HS were considered more brown when compared to steaks from steers fed LS. A

treatment × day interaction ($P = 0.06$) was detected for lightness values. At d 7, steaks from steers fed HS were considered lighter when compared to steaks from steers fed LS. Exposing steers to HS resulted in greater ($P \leq 0.08$) LM total conjugated linoleic acid (**CLA**) and 18:2*cis*9*trans*11 content when compared to the LM from steers fed LS, but the total CLA content decreased ($P = 0.09$) during 7-d of simulated RD. The total *n*-6 FA in the LM and proportion of dihomo-γ-linoleic (20:3*n*-6) in the LM and subcutaneous fat decreased ($P \leq 0.04$) when exposing steers to HS. Regardless of treatment, the proportion of LM capric, stearic, arachidic, palmitelaidic, total 18:1*trans*, total CLA, and total *trans* FA decreased ($P \leq 0.10$) after 7-d of simulated RD. The dietary inclusion of an additional 0.25% S provided from Na_2SO_4 in steer growing and finishing diets produced beef with greater CLA content without a concomitant decrease in RD shelf-life.

Key words: conjugated linoleic acid, beef cattle, fatty acid composition, shelf-life, sodium sulfate

INTRODUCTION

Compared to feed ingredients such as corn, corn dried distillers grains plus solubles (**DDGS**) contain greater amounts of polyunsaturated fatty acids (**PUFA**). In the rumen, unsaturated fatty acids (**USFA**) may be saturated by microorganisms via biohydrogenation (**BH**). Conjugated linoleic acid (**CLA**) is formed as a result of incomplete BH of linoleic acid. Studies have reported potential benefits of CLA for human health. Therefore, altering meat fatty acid (**FA**) composition to increase CLA content may have value to the beef industry.

Sulfuric acid added during ethanol production remains in DDGS to contribute to dietary S [0.8% S or greater, dry matter (**DM**) basis; Buckner et al., 2008]. Therefore, the prevalence of DDGS has led to research evaluating excess dietary S in cattle diets. Research has shown that

feeding corn distillers grains to cattle increases the concentration of PUFA in meat (Mello Jr. et al., 2012; Domenech-Pérez et al., 2017). It has been well established that beef with higher concentrations of PUFA is more likely to have increased lipid and myoglobin oxidation (Faustman et al., 2010). This is significant because lipid and myoglobin oxidation reduce beef color stability during retail display (**RD**). However, no information is available assessing excess dietary S using sodium sulfate (**Na_2SO_4**) in cattle diets on beef shelf-life and FA composition. Moreover, no information exists on the potential for beef FA composition to change during RD. It was hypothesized feeding greater S diets via supplemental Na_2SO_4 would increase beef CLA and other USFA content, and beef FA composition would change during RD. The objective of this study was to evaluate the effects of excess dietary S using supplemental Na_2SO_4 on beef shelf-life and FA composition.

MATERIALS AND METHODS

Animal handling procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (protocol #13008) and followed guidelines recommended in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010). Procedures involving human subjects were approved by the University of Arkansas Institutional Review Board (IRB #13-06-755).

Animals and experimental design

Twenty steers [initial body weight (**BW**) = 279 ± 13.2 kg] of predominantly Angus breeding were obtained from the University of Arkansas Cow-Calf Unit (Savoy, AR). Steers were stratified by initial BW and assigned randomly to 1 of 6 paddocks (3 to 4 steers/paddock) for a 114-d growing phase. Paddocks were assigned randomly to 1 of 2 dietary treatments: 1) low S ground corn and soybean meal supplement (**LS**) or 2) LS supplement with an additional

0.25% S provided from Na_2SO_4 (**HS**; Table 1). Steers grazed mixed grass (0.31% S, 17.8% crude protein, 26.8% acid detergent fiber, 57.1% neutral detergent fiber, and 10.4% ash, DM basis) paddocks (2.4-ha/paddocks) and were supplemented to meet NRC (2000) nutrient requirements for 0.15% S with a ground corn and soybean meal diet that did not contain any byproduct feeds (LS treatment). Remaining steers were offered an identical supplement to which an additional 0.25% S from Na_2SO_4 (Prince Agri Products, Inc., Quincy, IL) had been added in the total diet DM. Steers did not receive implants. In the event forage became limiting, steers were provided access to bermudagrass hay (0.36% S, 18.8% crude protein, 33.0% acid detergent fiber, 70.0% neutral detergent fiber, and 10.3% ash, DM basis) in quantities sufficient to ensure ad libitum access to forage. When the average BW of the steers reached 373 ± 0.2 kg, steers were stratified within dietary treatment by BW and assigned randomly to 16 dry-lot pens (1 to 2 steers/pen; 8 pens/dietary treatment). Steers remained on the same dietary treatment for a 123-d finishing phase. Steers on the LS treatment were offered a traditional corn and soybean meal finishing diet that did not contain any byproduct feeds or ionophore supplementation and met the NRC (2000) requirement of 0.15% S, whereas steers on the HS treatment steers were offered an identical diet except an additional 0.25% S was provided from Na_2SO_4 (Table 1). Comprehensive steer management and performance, carcass, and beef quality data are reported in a separate manuscript (Hawley et al., submitted for publication).

Slaughter and steak fabrication

All steers were harvested on the same date when the 12th-rib fat thickness was estimated to have reached 1 cm. Feed was withheld overnight with free access to water, and all steers were transported 351 km for harvest in a commercial abattoir (Creekstone Farms, Arkansas City, KS). Individual identification was maintained with each carcass. Following a 48-h chilling period, the

ribeye roll (Institutional Meat Purchase Specifications #112; USDA, 1996) was collected from both sides of the carcass during fabrication, vacuum-packaged, and transported on ice 372 km to the University of Arkansas Red Meat Abattoir (Fayetteville, AR). Ribeye rolls were aged 14 d (2°C) and fabricated by hand-cutting into 2.54-cm-thick longissimus muscle (**LM**) steaks. Starting at the caudal end of the rib section, 3 steaks were cut and used for the following analyses: proximate analysis, shelf-life, and FA composition analysis.

Proximate analysis

Moisture content was determined according to the freeze-drying method of Apple et al. (2001). Duplicate 15 g samples of minced LM were weighed, placed in 30 mL beakers, and reweighed. Beakers were then placed into vacuum flasks attached to the manifold of a freeze dryer (FreeZone 12L Freeze Dry System; Labconco Corp., Kansas City, MO) with a temperature setting of -50°C and a vacuum of less than 10 mm Hg. Samples were freeze-dried for 72 h, and beakers reweighed. Moisture percentage was calculated as the difference between the wet and freeze-dried sample weights divided by the wet weight. Freeze-dried samples were subsequently pulverized in a blender (Waring Product Division, Dynamics Corporation of America, New Hartford, CT) and analyzed in duplicate for ash (method 924.05; AOAC, 1990), protein (method 990.03; AOAC, 1995; Elementar Americas, Inc., Mt. Laurel, NJ), and ether extractable lipid (method 920.39; AOAC, 2000).

Shelf-life

Shelf-life was determined on LM samples from 2, 2.54-cm-thick steaks. Steaks were weighed, individually packaged on Styrofoam trays with absorbent pads, overwrapped with polyvinyl chloride film [oxygen transmission rate: 14,000 mL oxygen/(m^2·24 h) at 1 atmosphere; Koch Supplies Inc., Kansas City, MO], and randomly assigned to 0 or 7 d of simulated RD. Packaged steaks were then placed in open-topped, coffin-chest display cases (model LMG12;

Tyler Refrigeration Corp., Niles, MI) maintained at 3°C and displayed under 1,600 lx of continuous deluxe warm-white fluorescent lighting (40-W bulb, type F40T12; Phillips Inc., Somerset, NJ). Steaks were randomly assigned to location and had similar access to light. Steak display case position was redistributed daily from left to right and front to back to minimize any possible variation in temperature and light intensity.

Surface color measurement and drip loss. Visual discoloration of the LM in steaks was evaluated on d 0, 1, 4, and 7 of the simulated RD period by a trained 11-person panel. Panelist selection and training was conducted in accordance with American Meat Science Association guidelines (AMSA, 1991). Panel applicants were prescreened for color vision accuracy using a Farnsworth Munsell 100 Hue Test (Farnsworth, 1957). An error score of less than 60 was required for panel participation. Panelists evaluated the LM of each steak under display for total color and worst-point color (8-point scale; 1 = very bright red to 8 = tan to brown), and surface discoloration [6-point scale; 1 = no (0%) discoloration to 6 = extensive (81 to 100%) discoloration] in accordance with AMSA (2012) guidelines. Panelists were instructed to perform the evaluation at the same time each day to minimize variation.

Instrumental color of the LM in steaks under simulated RD was evaluated on d 0 (1 hr after packaging), 1, 4, and 7 using a Hunter Miniscan EZ Spectrophotometer (model 4500L; Hunter Associates Laboratory, Inc., Reston, VA) calibrated daily against black and white tiles. Color coordinate values of lightness (**L***), redness (**a***), and yellowness (**b***) were determined from the mean of 3 random readings collected from the cut LM surface of each steak using Illuminant A, 10º standard observer, and 2.54-cm aperture (CIE, 1976). Objective surface color measurements were also used to calculate: 1) chroma $[(a^{*2} + b^{*2})^{1/2}]$, a representation of the strength and weakness of chromatic color (greater values indicate a more vivid color); 2) hue

angle [$\tan^{-1}$(b*/a*)], a change from the true red axis, or a measurement of true redness (0° = true red to 90° = true yellow); 3) a*:b* (ratio of a* to b* color coordinates), an indicator of redness and discoloration (larger ratios indicate more redness and less discoloration); and 4) reflectance ratio (ratio of wavelengths 630 nm to 580), a change in red color (as the ratio increases, the sample is redder; AMSA, 2012). At the conclusion of the 7 d simulated RD period, steaks were removed from packages, blotted dry on paper towels, and weighed. The difference between the initial (d 0) and final (d 7) steak weight was divided by the initial steak weight then multiplied by 100 to calculate drip loss percentage. After surface color and drip loss measurements, steaks were vacuum-packaged and stored at -20°C until thiobarbituric acid reactive substances (**TBARS**) and FA composition analyses.

Thiobarbituric acid reactive substances. Thiobarbituric acid reactive substances assays were performed in duplicate on LM and subcutaneous fat (**SQ**) cross sections from d 0 and 7 simulated RD steaks in accordance to the procedure of Witte et al. (1970), with modifications described by Apple et al. (2001). Briefly, 2 g of minced sample was weighed into a 50 mL tube to which 8 mL of chilled 50 m*M* phosphate buffer (containing 0.1% ethylenediaminetetraacetic acid and 0.1% propyl gallate; pH 7.0) and 2 mL of 30% trichloroacetic acid was added, homogenized with a blender (Waring Product Division, Dynamics Corporation of America) for 30 s, and homogenate filtered through Whatman No. 4 filter paper (Whatman International Ltd., Maidstone, England) into 15 mL conical tubes. Then, 2 mL of the homogenate were combined with 2 mL of 0.02 *M* thiobarbuturic acid, and heated in a boiling water bath for 20 min. Samples were immediately cooled in an ice water bath for 15 min, and absorbance measured using a spectrophotometer (model UV-12015; Shimadzu Scientific Instruments, Inc., Columbia, MD) at

533 nm. Absorbance values were multiplied by a factor of 12.21 (Chamul, 2007) to attain TBARS values (mg malonaldehyde/kg of sample).

Fatty acid composition analysis

Samples of LM and SQ were removed from d 0 and 7 simulated RD steaks, freeze-dried for 72 h (FreeZone 12L Freeze Dry System; Labconco Corp.) with a temperature setting of ¯50°C and a vacuum of less than 10 mm Hg, and pulverized with a blender (Waring Product Division, Dynamics Corporation of America) before duplicate 175-mg samples were subjected to transesterification. Feed samples were dried at 50°C in a forced air oven and ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) through a 1-mm screen before duplicate 225-mg samples were subjected to transesterification. Fatty acid methyl esters were formed by base-catalyzed transesterification in accordance with the procedure of Murrieta et al. (2003). Briefly, 1 mL of hexane containing 0.5 mg/mL of an internal standard [glyceryl tridecanoic acid (13:0)] was added to 16 × 125 mm screw-cap tubes. Hexane was evaporated under a hood to concentrate the internal standard. Ground samples were weighed into to the prepared tubes and incubated in 2 mL of 0.2 *M* methanolic potassium hydroxide at 50°C for 30 min with vortex mixing 2 to 3 times/min until samples were dissolved. Tubes were cooled to room temperature, and 1 mL of saturated sodium chloride and 2 mL of highly purified hexane were added to each tube. Tubes were subsequently vortexed and centrifuged (1,100 × *g*, 22°C for 20 min) to separate phases. Fatty acid methyl esters (**FAME**) in the hexane layer (approximately 1 mL) were transferred to gas chromatography vials that contained a 1 mm bed of anhydrous Na_2SO_4. Separation of FAME was achieved by gas chromatography (model HP 5890 Series II; Agilent Technologies, Inc., Wilmington, DE) with a 0.25 mm × 100 m capillary column (model SP-2560; Supelco Inc., Bellefonte, PA) and He as a carrier gas (20 mL/s) with a 60:1 split ratio. Oven temperature was maintained at 150°C for 5 min, ramped at 4°C/min to 194°C for 15 min, and then ramped at

2.5°C/min to 235°C for 16.25 min. Injector and detector temperatures were maintained at 250°C. Identification of FAME peaks was accomplished using individual acids (Nu-Chek Prep, Elysian, MN; Matreya, Pleasant Gap, PA) and purified standards (Supelco 37 Component FAME Mix; Supelco Inc.) purified standards. The FA compositions were calculated using the peak areas and expressed on a weight percentage basis. Fatty acid composition was unavailable for multiple d 7 samples owing to a freeze-dryer malfunction.

Statistical analysis

Proximate composition and shelf-life data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Inst., Inc., Cary, NC) by means of a first-order autoregressive covariance structure. Denominator degrees of freedom were estimated using the Kenward-Rogers option. Adherence of the data to the assumptions of the statistical test was established. Proximate composition data were analyzed as randomized designs. Dietary treatment was the fixed effect and pen (experimental unit) was included as the random effect in the models. Surface color measurements were analyzed as repeated measures. Fixed effects included in the model were dietary treatment and day, as well as the 2-way interaction, whereas pen (experimental unit) was included as a random effect. Day was included as a repeated effect. Fatty acid composition data were analyzed as repeated measures using the MIXED procedure of SAS by means of a first-order autoregressive covariance structure. Denominator degrees of freedom were estimated using the Kenward-Rogers approximation. The fixed (main) effects in the model included treatment and day, as well as the 2-way interaction. Pen was included in the model as a random effect and experimental unit. Day was included as a repeated effect. Least squares means were partitioned at the 10% level of significance by way of the probability of differences option. Statistical significance was declared at $P \leq 0.10$.

RESULTS AND DISCUSSION

The objective of this study was to evaluate the effects of excess dietary S using supplemental Na_2SO_4 on beef shelf-life and FA composition. It was hypothesized that feeding greater S diets via supplemental Na_2SO_4 would increase beef CLA and other USFA content, and beef FA composition would change during RD. These data suggest exposing growing-finishing cattle to greater S diets via supplemental dietary S from Na_2SO_4 has no impact on beef proximate composition and limited impact on beef shelf-life and FA composition. However, when interpreting the results of this study, there are a couple factors that should be considered. Greater steer numbers would increase the power for detection of beef proximate composition, shelf-life, and FA composition differences. In light of the small sample size per treatment group, the power to detect significant differences was limited and a factor in the choice to use a type I error rate of 0.10. Moreover, the lack of studies that used Na_2SO_4 for comparison prohibited establishing a substantiated relationship between treatment and outcome.

Proximate analysis

Feeding steers excess dietary S via supplemental Na_2SO_4 did not ($P \geq 0.44$; Table 3) influence LM proximate composition. No difference in fat content was expected as marbling scores were consistent ($P = 0.51$; data not shown) between treatments. Additional information regarding the impact of dietary Na_2SO_4 on beef proximate composition does not exist in literature. Similarly, while DDGS are readily available as an ethanol byproduct for cattle feed, there is little information on their impact as a dietary S source on beef proximate composition in literature. However, one study examined feeding steers DDGS at 18 or 40% DM (0.22 or 0.34% S, respectively) in corn-based diets (Pogge et al., 2014). Steers fed a 40% DDGS diet [1.3 Mcal net energy for gain (**NEg**)/kg DM] experienced increased longissimus thoracis moisture content when compared to steers fed a 18% DDGS diet (1.3 Mcal NEg/kg DM), whereas longissimus

thoracis fat and protein content were consistent between treatments. These findings suggest that while dietary S source may be an important characteristic that influences rumen metabolism, it may not readily influence beef proximate composition. The more important aspect is likely dietary energy concentration. In the current study, calculated NEg (Vasconcelos and Galyean, 2008) did not differ ($P = 0.16$) by treatment (1.02 and 1.06 Mcal NEg/kg DM; LS and HS treatment, respectively). Accordingly, supplemental Na_2SO_4 was not anticipated to influence LM proximate composition. Conversely, Buttrey et al. (2013) reported LM steaks from steers fed 35% wet corn distillers grains with solubles (**WDGS**; 0.34% S) had decreased moisture and tended to have greater total fat content compared to LM steaks from steers fed a 0% WDGS (0.12% S) dry-rolled corn-based control diet. The NEg of the 35% WDGS diet was greater than the diet without WDGS (1.34 and 1.25 Mcal NEg/kg DM, respectively).

Shelf-life

Thiobarbituric acid reactive substances. Lipid oxidation, as indicated by TBARS concentration, suggests that excess dietary S supplementation from Na_2SO_4 does not influence shelf-life. Thiobarbituric acid reactive substances increased ($P < 0.0001$; Fig. 1) over time as expected; however, no differences ($P > 0.42$) were found among steaks from different dietary treatments. These results contradict those of previous studies (Mello Jr. et al., 2012; Buttrey et al., 2013; Ribeiro et al., 2018) that reported distillers grains impacted the TBARS concentration in beef. Buttrey et al. (2013) compared WDGS in steam-flaked corn based finishing diets at 0 or 30% of diet DM (0.12 and 0.34% S; 5.42 and 5.58 Mcal NEg/kg DM, respectively) and WDGS in dry-rolled corn based finishing diets at 0 or 30% of diet DM (0.12 and 0.34% S; 5.05 and 5.35 Mcal NEg/kg DM, respectively). Loin steaks from steers fed 30% WDGS exhibited greater oxidation after 7 d of RD when compared with 0%. Conversely, others have reported no

differences in TBARS in beef from cattle fed dry-rolled and steam-flaked corn-based diets containing 0 to 75% DG of diet DM (Gill et al., 2008; Depenbusch et al., 2009; Gunn et al., 2009). These findings suggest that dietary S source may be an important characteristic that influences lipid oxidation. Greater PUFA deposition is regularly observed in muscular tissue when corn distillers grains are substituted for corn (Mello Jr. et al., 2012; Veracini et al., 2013; Domenech-Pérez et al., 2017; Ribeiro et al., 2018). Zhang et al. (2007) demonstrated that PUFA are more easily oxidized when compared with USFA. Therefore, the greater PUFA content in steaks from cattle fed corn distillers grains is likely subjected to greater lipid oxidation when compared with steaks from cattle fed Na_2SO_4.

Oxidation can be controlled by the amount of antioxidant compounds found in muscle tissue (Calkins and Hodgen, 2007). In pork, an increase in dietary S has been associated with an increase in S containing amino acids which are known for their antioxidant capabilities (Song et al., 2013). This may explain why the HS diet in this study exhibited numerically lower TBARS values when compared to the LS diet after 7-d of simulated RD. Lipid oxidation that occurs during storage can diminish beef color, flavor, aroma, and consequently, shelf-life (Ladeira et al., 2014). Campo et al. (2006) concluded that a TBARS concentration of 2.28 mg/kg could be considered a limiting threshold for oxidized beef acceptability. In this study, beef remained below this limiting threshold after 7 d under RD conditions regardless of dietary treatment.

Surface color measurement and drip loss. Instrumental and visual color profiles suggest that excess dietary S supplementation from Na_2SO_4 has minimal impact on RD shelf-life. A treatment × day interaction ($P < 0.01$) was detected for worst-point color when evaluated by a trained visual panel (Table 4). At d 4, steaks from steers fed HS were considered more brown when compared to steaks from steers fed LS. No differences ($P = 0.32$) were observed among

treatments for total color; however, total color based on a visual score increased over time ($P <$ 0.0001). These findings were corroborated by similar a* ($P = 0.18$) and hue angle ($P = 0.26$) values between treatments. Redness scores in the current study confirm findings by Zerby et al. (1999) who reported that visual redness scores were moderately to highly correlated to instrumental a* values. In contrast, others have observed decreased beef color stability when evaluating varying inclusions of corn distillers grains (Depenbusch et al., 2009; Leupp et al., 2009; Mello Jr. et al., 2012; Veracini et al., 2013; Ribeiro et al., 2018). Veracini et al. (2013) reported a significant decrease in redness on d 7 as level of reduced-fat modified WDGS increased from 0 to 70% of diet DM (0.14 to 0.53% S; 3.45 to 6.54% crude fat, respectively). Myoglobin is the protein responsible for the bright cherry red color of beef. Lipid oxidation enhances myoglobin oxidation due to oxidation propagation by PUFA (Faustman et al., 2010). Increased PUFA content due to corn distillers grains is associated with reduced beef color attributes (Ribeiro et al., 2018). Consequently, the reduced color stability of beef from cattle supplemented corn distillers grains is likely due to oxidation propagation caused by greater PUFA content when compared with beef from cattle fed Na_2SO_4.

No differences ($P = 0.30$) were observed among treatments for visual steak discoloration; however, percent discoloration based on a visual score increased over time ($P <$ 0.0001). Hood and Riordan (1973) reported a significant decline in purchasing decision with 20% surface discoloration on RD beef to result in sale reductions of up to 50%. Data from the current study suggests that by d 4, the 20% discoloration threshold was met by steaks from both treatments. A treatment × day interaction ($P = 0.06$) was detected for L* values (Table 5). At d 7, steaks from steers fed HS were considered lighter when compared to steaks from steers fed LS. All color coordinate values gradually decreased ($P <$ 0.0001) over the simulated RD period. The gradual

decrease in objective color evaluation was expected due to fresh beef color deterioration caused by lipid oxidation. No differences in drip loss were identified among treatments (P = 0.97; 5.1 and 5.1%; LS and HS treatment, respectively).

Fatty acid composition

Excess dietary S supplementation from Na_2SO_4 may favorably modify steer LM FA composition to make beef more healthful. Exposing steers to HS resulted in greater (P = 0.08) LM total CLA content when compared to the LM from steers fed LS, but the total CLA content decreased (P = 0.09; Table 6) during 7-d of simulated RD. Biohydrogenation is the conversion of dietary USFA to SFA via isomerization and hydrogenation by rumen bacteria (Nafikov and Beitz, 2007). Biohydrogenation was originally proposed as an alternative ruminal H_2 sink to methanogenesis or propionigenesis (Lennarz, 1966). Thus, the increase in LM total CLA content from feeding HS suggests some of the hydrogen available for BH may have been used for the reduction of Na_2SO_4 to sulfide by sulfate reducing bacteria and the subsequent production of H_2S from sulfide (Knight et al., 2008; Cammack et al., 2010).

The primary CLA isomers, 18:2*cis*9*trans*11 and 18:2*trans*10*cis*12, have different biological effects. Production practices that increase the formation and absorption of 18:2*trans*10*cis*12 from the digestive system depress adipose tissue development (Mersmann, 2002; Smith et al., 2009), whereas the formation and absorption of 18:2*cis*9*trans*11 is associated with an anticarcinogenic effect (McGuire and McGuire, 2000). Exposing steers to HS resulted in greater (P = 0.07) LM 18:2*cis*9*trans*11 content compared to the LM from steers fed LS. Thus, the consumption of beef enriched with 18:2*cis*9*trans*11 from Na_2SO_4 supplementation may be an alternative to reduce chronic diseases. The shift in FA profile also suggests that dietary Na_2SO_4 may have a positive influence on BH. In the normal BH pathway, linoleic acid (18:2*n*-6) is isomerized to 18:2*cis*9*trans*11 and reduced to vaccenic acid (18:1*trans*11; Palmquist et al.,

2005). However, when the rumen environment is altered by finishing diets, an alternative BH pathway occurs to produce 18:2*trans*10*cis*12 and isooleic acid (18:1*trans*10). The greater (P = 0.07) LM 18:2*cis*9*trans*11 content from feeding steers HS suggests that dietary Na_2SO_4 had a positive impact on BH by promoting the normal BH pathway.

The total *n*-6 FA in the LM and proportion of dihomo-γ-linoleic (20:3*n*-6) in the LM and SQ decreased ($P \leq 0.04$; Tables 6 and 7) when exposing steers to HS. Long chain PUFA synthesis involves a series of desaturation and elongation reactions starting with the essential precursors 18:2*n*-6 and α-linolenic (18:3*n*-3) acids for the *n*-6 and *n*-3 pathways, respectively. In the current study, the effect of Na_2SO_4 supplementation on LM 18:2*n*-6 content approached statistical significance (P = 0.11; 2.20 and 2.05%; LS and HS treatment, respectively). Therefore, the difference in essential 18:2*n*-6 precursor availability could be the reason for lower LM total *n*-6 FA content in steers fed HS when compared to steers fed LS.

Although contested, the ratios of product to precursor FA concentrations are often used as indices for enzyme activities (desaturases and elongases) involved in *n*-3 and *n*-6 PUFA metabolism (Zhang et al., 2007; Ntawubizi et al., 2010). Elongase catalyzes the conversion of γ-linolenic (18:3*n*-6) to 20:3*n*-6. The elongase index (20:3*n*-6/18:3*n*-6), an indicator of the elongase influence on the overall synthesis of 20:3*n*-6, was lower ($P < 0.10$) in the LM from steers fed HS compared to steers fed LS (0.77 and 0.99; HS and LS steers, respectively). Accordingly, the lower LM 20:3*n*-6 content in steers fed HS could be attributed to decreased LM elongase activity. Similarly, it was anticipated that the lower SQ 20:3*n*-6 content in steers fed HS could be attributed to decreased SQ elongase activity; however, the SQ elongase index did not differ (P = 0.80) between dietary treatments. These results may be owing to an insufficient number of SQ samples to detect a treatment difference.

Beef FA composition results in the current study differ from those reported by others when replacing corn with corn distillers grains in beef cattle finishing diets. These studies report beef from cattle fed finishing diets containing corn distillers grains compared with cattle fed corn-based finishing diets exhibit increased proportions of total PUFA in general and 18:2*n*-6, CLA, or total *n*-6 in particular (Gill et al., 2008; Depenbusch et al., 2009; Koger et al., 2010; Schoonmaker et al., 2010; Mello Jr. et al., 2012; Veracini et al., 2013; Domenech-Pérez et al., 2017; Ribeiro et al., 2018). Biohydrogenation occurs following lipolysis and is dependent upon ruminal pH (Kalscheur et al., 1997; Fuentes et al., 2009, 2011). This suggests that the inclusion of corn distillers grains in cattle diets would produce a more acidic ruminal environment when compared to dietary Na_2SO_4 to suppress BH, thereby increasing the availability and tissue deposition of USFA in beef. It is important to note that most rumen metabolic processes are affected by others, with BH being particularly dependent on other factors. Vander Pol et al. (2009) reported greater 18:2*n*-6 content in the duodenum of steers fed WDGS (0.36% S; 7.19% crude fat) compared with steers fed a control corn-based finishing diet (0.16% S; 3.93% crude fat); however, mean ruminal pH did not differ between treatments. Accordingly, the increased availability and tissue deposition of USFA in beef of cattle fed corn distillers grains when compared to dietary Na_2SO_4 may also be due to dietary PUFA offering some degree of protection from BH or inhibiting ruminal fibrolytic bacteria such as *Butyrivibrio fibrisolvens*, the main ruminal BH bacterium (Yang et al., 2009).

Fatty acid composition during simulated RD

Changing FA composition during RD may alter the healthfulness of beef. Differences in the proportion of individual FA during simulated RD suggest the oxidative processes in meat that involve the formation of unstable free radicals. A treatment × day interaction ($P = 0.09$) was detected for LM 18:1*trans*11. At d 0, there were no differences ($P = 0.67$) in LM 18:1*trans*11

concentrations among treatment groups. However, after 7-d of simulated RD, LM 18:1*trans*11 concentrations were greater ($P = 0.08$) from steers fed LS when compared to the LM from steers fed HS. Regardless of treatment, the proportion of LM capric (10:0), stearic (18:0), arachidic (20:0), palmitelaidic (16:1*trans*), total 18:1*trans*, total CLA, and total *trans* FA decreased ($P \leq 0.10$) after 7-d of simulated RD. Also, the proportion of SQ 10:0, myristoleic (14:1), total CLA, arachidonic (20:4*n*-6), and total *n*-3 FA decreased ($P \leq 0.09$), whereas the proportion of SQ 18:0 increased ($P = 0.02$), after 7-d of simulated RD regardless of treatment. Thermodynamically unfavorable free radicals likely reacted with other molecules to reach a more stable state by cleaving hydrogen atoms from carbon-hydrogen bonds and donating or accepting electrons from neighboring compounds to result in the chemical modification of surrounding FA (Halliwell and Gutteridge, 2015). Thus, if the oxidative processes of meat can be controlled during RD, it may be possible to preserve the healthfulness of beef by maintaining the USFA composition in general and total CLA in particular.

In conclusion, the dietary inclusion of an additional 0.25% S provided from Na_2SO_4 in steer growing and finishing diets produced beef with greater CLA content without a concomitant decrease in RD shelf-life. The shift in FA profile also suggests that dietary Na_2SO_4 may have a positive influence on BH. Changing FA composition during RD may alter the healthfulness of beef. Thus, if the oxidative processes of meat can be controlled during RD, it may be possible to preserve the healthfulness of beef by maintaining the USFA composition in general and total CLA in particular.

ACKNOWLEDGMENTS

This research was supported by the Arkansas Beef Council. The authors thank T. Johnson (University of Arkansas, Department of Animal Science, Fayetteville, AR) and A. Ryan

(Renaissance Nutrition, Inc., Roaring Spring, PA) for assistance with meat fabrication and data collection.

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Table 1. Ingredient and analyzed composition of corn and soybean meal diets fed to steers.

	Treatments			
	Growing phase[1,2]		Finishing phase[3]	
Item	LS	HS	LS	HS
Ingredient, % as-fed basis				
Corn grain, cracked	81.2	80.4	76.3	76.1
Bermudagrass hay	--	--	7.0	7.0
Soybean meal	12.6	12.6	6.0	6.0
Cottonseed hulls	--	--	5.0	5.0
Molasses, cane	1.8	1.8	3.0	3.0
Calcium carbonate	0.45	0.45	1.0	1.0
Salt	3.30	0.00	0.85	0.00
Sodium sulfate, anhydrous[4]	0.00	4.10	0.00	1.05
Urea	--	--	0.80	0.80
Trace mineral premix[5]	0.10	0.10	0.025	0.025
Vitamin A, D, and E[6]	0.10	0.10	0.05	0.05
Rumensin premix[7]	0.40	0.40	--	--
Analyzed composition,[8] % DM basis				
CP	14.9	14.5	14.3	15.1
ADF	2.1	2.0	6.4	6.6
NDF	16.5	17.2	23.7	27.7
S	0.30	1.31	0.19	0.42

[1]Fed at a rate of 1.8 $kg \cdot d^{-1} \cdot hd^{-1}$ to cattle grazing mixed grass pasture and also offered ad libitum access to bermudagrass hay.
[2]LS = low S, 0.31% total dietary S and HS = high S, 0.58% total dietary S.

Table 1 (Cont).
[3]LS = low S, 0.19% total dietary S and HS = high S, 0.42% total dietary S.
[4]Prince Agri Products, Inc., Quincy, IL.
[5]Trace mineral premix composition (mg/kg): 500 Co; 40,000 Cu; 2,000 I; 10,000 Fe; 80,000 Mn; 600 Se; and 120,000 Zn (Nutrablend, Neosho, MO).
[6]Vitamin premix composition (IU/kg): 1,816,000 vitamin A; 36,320 vitamin D; and 227 vitamin E.
[7]Rumensin 80 (Elanco, Indianapolis, IN) supplied 22 mg of monensin/kg of diet DM.
[8]CP = crude protein; ADF = acid detergent fiber; and NDF = neutral detergent fiber.

Table 2. Fatty acid (FA) composition of corn and soybean meal diets fed to steers (as-fed basis).

	Treatment			
	Growing phase[1]		Finishing phase[2]	
FA composition,[3] %	LS	HS	LS	HS
No. of samples	2	2	2	2
SFA				
Total[4]	18.82	18.47	17.84	17.57
Myristic (14:0)	ND[5]	ND	ND	0.069
Palmitic (16:0)	15.29	15.03	14.72	14.44
Stearic (18:0)	2.83	2.77	2.46	2.48
Arachidic (20:0)	0.70	0.67	0.66	0.58
MUFA				
Total[6]	30.28	29.89	26.82	27.33
Palmitoleic (16:1*cis*)	0.08	ND	0.09	0.18
Total 18:1*trans* FA	0.08	ND	ND	ND
Oleic (18:1*cis*9)	29.88	29.65	25.60	25.16
Vaccenic (18:1*trans*11)	ND	ND	0.89	1.75
Gadoleic (20:1*cis*11)	0.24	0.24	0.24	0.24
PUFA				
Total[7]	48.80	50.12	54.41	54.30
Linoleic (18:2*n*-6)	46.44	47.78	52.41	52.32
α-Linolenic (18:3*n*-3)	1.26	1.37	1.37	1.38
Eicosapentaenoic (20:5*n*-3)	0.26	0.25	0.25	0.27

Table 2 (Cont.)

	Treatment			
	Growing phase[1]		Finishing phase[2]	
FA composition,[3] %	LS	HS	LS	HS
Docosahexaenoic (22:6*n*-3)	0.84	0.72	0.38	0.33

Values represent the mean value (n = 2 per treatment).
[1]LS = low S, 0.31% total dietary S and HS = high S, 0.58% total dietary S.
[2]LS = low S, 0.19% total dietary S and HS = high S, 0.42% total dietary S.
[3]Amount of FA in sample expressed on a weight percentage basis as determined by gas chromatography.
[4]Total saturated fatty acids (SFA) = 14:0, 16:0, 18:0, and 20:0.
[5]ND = not detected.
[6]Total monounsaturated fatty acids (MUFA) = 16:1*cis*, total 18:1*trans* FA, 18:1*cis*9, 18:1*cis*11, and 20:1*cis*11.
[7]Total polyunsaturated fatty acids (PUFA) = 18:2*n*-6, 18:3*n*-3, 20:5*n*-3, and 22:6*n*-3.

Table 3. Least square means for the effects of excess dietary S from supplemental sodium sulfate fed to growing-finishing steers on longissimus muscle proximate composition.

	Treatment[1]			
Item, %	LS	HS	SEM	*P*-value
Pens (steers)	8 (10)	8 (10)		
Moisture	68.97	69.22	0.56	0.76
Total fat	16.01	15.54	1.51	0.83
Crude protein	76.58	78.14	2.45	0.66
Ash	3.40	3.57	0.15	0.44

[1]LS = low S and HS = high S.

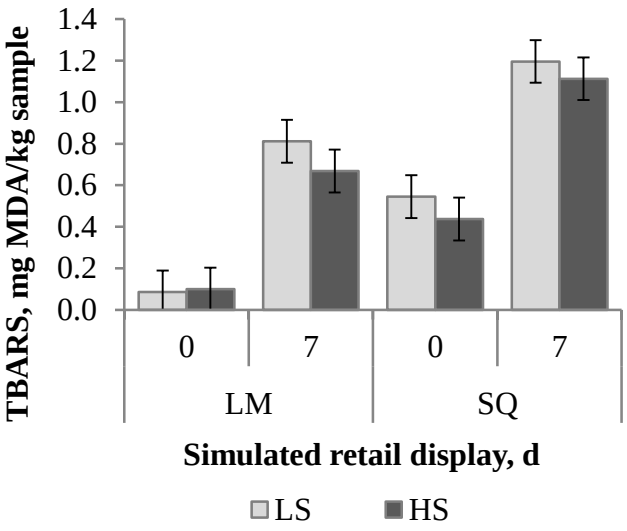

Figure 1. Least square means for the effects of excess dietary S from supplemental sodium sulfate fed to growing-finishing steers on d 0 and 7 simulated retail display steak longissimus muscle (LM; day effect, $P < 0.0001$; SEM = 0.09) and subcutaneous fat (SQ; day effect, $P < 0.0001$; SEM = 0.12) thiobarbituric acid reactive substances (TBARS). MDA = malondialdehyde; LS = low S; and HS = high S.

Table 4. Least square means for the effects of excess dietary S from supplemental sodium sulfate fed to growing-finishing steers on simulated retail display steak longissimus muscle visual panel (n = 11) ratings.

	Treatment[1]											
	d 0		d 1		d 4[2]		d 7			*P*-values[3]		
Characteristic[4]	LS	HS	LS	HS	LS	HS	LS	HS	SEM	Trt	Day	Trt × Day
Total color	1.63	1.75	2.16	2.19	3.50	3.98	7.29	7.24	0.18	0.32	<0.0001	0.35
Worst-point color	1.80	1.80	2.38	2.62	6.38[a]	7.70[b]	7.69	7.71	0.21	0.05	<0.0001	<0.01
Surface discoloration	1.17	1.16	1.22	1.28	2.14	2.53	5.68	5.66	0.12	0.30	<0.0001	0.25

[1]LS = low S and HS = high S.
[2]n = 4 panelists owing to a federal holiday.
[3]*P*-values for the effect of dietary treatment (Trt), day (Day), and their interaction (Trt × Day).
[4]Steaks were evaluated for total color and worst-point color score on an 8-point psychometric scale where 1 = very bright red; 2 = bright red; 3 = dull red; 4 = slightly dark red; 5 = moderately dark red; 6 = dark red to dark reddish tan; 7 = tannish red; and 8 = tan to brown; AMSA, 2012). Worst-point color was evaluated as about the size of a dime. Steaks were evaluated for percent discoloration on a 6-point scale where 1 = no (0%) discoloration; 2 = slight (1 to 20%) discoloration; 3 = small (21 to 40%) discoloration; 4 = modest (41 to 60%) discoloration; 5 = moderate (61 to 80%) discoloration; and 6 = extensive (81 to 100%) discoloration; AMSA, 2012).
[a,b]Means with no common superscripts within the same simulated retail display time differ ($P \leq 0.10$).

Table 5. Least square means for the effects of excess dietary S from supplemental sodium sulfate fed to growing-finishing steers on simulated retail display steak longissimus muscle objective color characteristics.[1]

	Treatment[2]											
	d 0		d 1		d 4		d 7			*P*-values[3]		
Characteristic	LS	HS	LS	HS	LS	HS	LS	HS	SEM	Trt	Day	Trt × Day
Redness (a*)	33.64	32.73	30.37	30.34	25.26	24.05	13.27	11.71	0.72	0.18	<0.0001	0.67
Yellowness (b*)	26.08	25.36	23.97	23.92	20.77	20.40	16.19	16.10	0.41	0.40	<0.0001	0.76
Lightness (L*)	46.37	45.92	44.19	44.98	42.76	42.26	40.38[a]	41.12[b]	0.72	0.88	<0.0001	0.06
Chroma[4]	42.5 6	41.41	38.70	38.64	32.72	31.59	21.03	19.94	0.76	0.22	<0.0001	0.77
Hue angle[5]	52.23	52.25	51.70	51.72	50.35	49.24	38.88	35.96	0.84	0.26	<0.0001	0.26
a*:b*[6]	1.29	1.29	1.27	1.27	1.21	1.17	0.82	0.73	0.03	0.25	<0.0001	0.26
Reflectance ratio[7]	7.02	6.85	5.82	5.79	4.55	4.04	1.40	1.23	0.22	0.30	<0.0001	0.64

[1]a* values are a measure of redness (positive values = red, negative values = green); b* values are a measure of yellowness (positive values = yellow, negative values = blue); and L* values measure darkness to lightness (0 = black, 100 = white).
[2]LS = low S and HS = high S.
[3]*P*-values for the effect of dietary treatment (Trt), day (Day), and their interaction (Trt × Day).

Table 5 (Cont.)

[4]Chroma = $(a^{*2} + b^{*2})^{1/2}$; values represent the strength and weakness of chromatic color (greater values indicate a more vivid color; i.e., red intensity).

[5]Hue angle = $\tan^{-1}(b^*/a^*)$; values represent the change from the true red axis, or a measurement of true redness (0° = true red to 90° = true yellow).

[6]a*:b* = ratio of a* to b* color coordinates; values represent redness and discoloration (larger ratios indicate more redness and less discoloration).

[7]Reflectance ratio = ratio of wavelengths 630 nm to 580 nm; values represent the change in red color (as the ratio increases, the sample is redder).

[a,b]Means with no common superscripts within the same simulated retail display time differ ($P \leq 0.10$).

Table 6. Least square means for the effects of excess dietary S from supplemental sodium sulfate fed to growing-finishing steers on d 0 and 7 simulated retail display steak longissimus muscle fatty acid (FA) composition.

	Treatment[1]							
	d 0		d 7			*P*-value[2]		
FA composition,[3] %	LS	HS	LS	HS	SEM	Trt	Day	Trt × Day
No. of samples[4]	10	10	5	8				
SFA								
Total[5]	45.90	45.75	45.21	45.89	0.608	0.75	0.41	0.21
Capric (10:0)	0.031	0.038	0.017	0.014	0.008	0.83	0.04	0.55
Lauric (12:0)	0.043	0.048	0.035	0.046	0.010	0.47	0.59	0.76
Myristic (14:0)	2.79	2.68	2.76	2.72	0.168	0.75	0.89	0.60
Pentadecanoic (15:0)	0.37	0.37	0.37	0.37	0.023	0.84	0.97	0.89
Palmitic (16:0)	27.52	26.87	27.47	27.25	0.469	0.51	0.42	0.29
Margaric (17:0)	1.13	1.08	1.12	1.07	0.042	0.40	0.85	0.97
Stearic (18:0)	13.94	14.67	13.34	14.31	0.460	0.18	0.07	0.63
Arachidic (20:0)	0.077	0.085	0.073	0.076	0.004	0.30	0.10	0.54

Table 6 (Cont.)

	Treatment[1]							
	d 0		d 7			*P*-value[2]		
FA composition,[3] %	LS	HS	LS	HS	SEM	Trt	Day	Trt × Day
MUFA								
Total[6]	47.02	47.22	47.48	47.19	0.680	0.96	0.41	0.35
Myristoleic (14:1)	0.66	0.63	0.69	0.67	0.062	0.77	0.27	0.90
Palmitelaidic (16:1*trans*)	0.25	0.28	0.24	0.25	0.011	0.17	0.05	0.49
Palmitoleic (16:1*cis*)	3.46	3.22	3.61	3.34	0.134	0.15	0.14	0.88
Heptadecenoic (17:1*trans*)	0.099	0.111	0.092	0.104	0.007	0.16	0.17	0.91
Total 18:1*trans* F A	1.61	1.73	1.46	1.58	0.096	0.35	0.05	0.98
Oleic (18:1*cis*9)	38.40	38.68	38.77	38.78	0.736	0.89	0.38	0.59
Vaccenic (18:1*trans*11)	2.35	2.39	2.51[a]	2.25[b]	0.067	0.08	0.94	0.09
Gadoleic (20:1*cis*11)	0.18	0.18	0.17	0.17	0.013	0.92	0.26	0.80
PUFA								
Total[7]	4.19	4.03	4.67	3.91	0.287	0.14	0.54	0.32
Linoleic (18:2*n*-6)	2.20	2.05	2.47	2.03	0.166	0.11	0.45	0.39

Table 6 (Cont.)

	Treatment[1]							
	d 0		d 7			*P*-value[2]		
FA composition,[3] %	LS	HS	LS	HS	SEM	Trt	Day	Trt × Day
Total CLA[8]	0.35	0.43	0.28	0.36	0.039	0.08	0.09	0.93
18:2*cis*9*trans*11	0.29	0.33	0.27	0.32	0.019	0.07	0.28	1.00
18:2*trans*10*cis*12	ND[9]	ND	ND	ND				
18:2*cis*9*cis*11	0.014	0.023	0.004	0.011	0.006	0.11	0.09	0.89
18:2*trans*9*trans*11	0.008	0.015	ND	0.004	0.004	0.17	0.06	0.83
α-Linolenic (18:3*n*-3)	0.20	0.22	0.21	0.21	0.013	0.44	0.60	0.34
γ-Linolenic (18:3*n*-6)	0.20	0.22	0.21	0.21	0.013	0.44	0.59	0.34
Eicosadienoic (20:2)	0.020	0.015	0.010	0.006	0.006	0.52	0.12	0.97
Dihomo-γ-linoleic (20:3*n*-6)	0.20	0.17	0.24	0.18	0. 020	0.02	0.27	0.45
Arachidonic (20:4*n*-6)	0.53	0.47	0.67	0.48	0.073	0.12	0.31	0.37
Eicosapentaenoic (20:5*n*-3)	0.20	0.19	0.21	0.19	0.011	0.16	0.77	0.87
Docosapentaenoic (22:5*n*-3)	0.27	0.25	0.33	0.25	0.034	0.15	0.30	0.41
Docosahexaenoic (22:6*n*-3)	0.013	0.018	0.013	0.003	0.007	0.74	0.15	0.17

Table 6 (Cont.)

	Treatment[1]							
	d 0		d 7			*P*-value[2]		
FA composition,[3] %	LS	HS	LS	HS	SEM	Trt	Day	Trt × Day
Total unsaturated FA[10]	51.21	51.25	51.80	51.26	0.621	0.76	0.42	0.43
Total *trans* FA[11]	1.97	2.12	1.79	1.93	0.108	0.29	0.05	0.91
Total *n*-6 FA[12]	3.13	2.92	3.62	2.89	0.259	0.09	0.39	0.35
Total *n*-3 FA[13]	0.68	0.68	0.76	0.65	0.051	0.28	0.57	0.34
n-6:*n*-3[14]	4.62	4.31	4.65	4.40	0.157	0.19	0.48	0.69
PUFA:SFA[15]	0.091	0.089	0.103	0.085	0.006	0.12	0.57	0.27
Unsaturated FA:SFA[1 6]	1.12	1.12	1.15	1.12	0.029	0.73	0.45	0.34

[1]LS = low S and HS = high S.
[2]*P*-values for the effect of dietary treatment (Trt), day (Day), and their interaction (Trt × Day).
[3]Amount of FA in sample expressed on a weight percentage basis as determined by gas chromatography.
[4]Fatty acid composition unavailable for multiple d 7 samples owing to a freeze dryer malfunction. Samples were subjected to transesterification in duplicate.
[5]Total saturated fatty acids (SFA) = 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0.
[6]Total monounsaturated fatty acids (MUFA) = 14:1, 16:1*trans*, 16:1*cis*, 17:1*trans*, total 18:1*trans* FA, 18:1*cis*9, 18:1*cis*11, and 20:1*cis*11.
[7]Total polyunsaturated fatty acids (PUFA) = 18:2*n*-6, total CLA, 18:3*n*-3, 18:3*n*-6, 20:2, 20:3*n*-6, 20:4*n*-6, 20:5*n*-3, 22:5*n*-3, and 22:6*n*-3.
[8]Total conjugated linoleic acid (CLA) = 18:2*cis*9*trans*11, 18:2*trans*10*cis*12, 18:2*cis*9*cis*11, and 18:2*trans*9*trans*11.
[9]ND = not detected.

Table 6 (Cont.)

[10]Total unsaturated FA = total MUFA and total PUFA.

[11]Total *trans* FA = 16:1*trans*, 17:1*trans*, and total 18:1*trans* FA.

[12]Total *n*-6 FA = 18:2*n*-6, 18:3*n*-6, 20:3*n*-6, and 20:4*n*-6.

[13]Total *n*-3 FA = 18:3*n*-3, 20:5*n*-3, 22:5*n*-3, and 22:6*n*-3.

[14]*n*-6:*n*-3 = total *n*-6 FA/total *n*-3 FA.

[15]PUFA:SFA = total PUFA/total SFA.

[16]PUFA:SFA = total unsaturated FA/total SFA.

[a,b]Means with no common superscripts within the same simulated retail display time differ ($P \leq 0.10$).

Table 7. Least square means for the effects of excess dietary S from supplemental sodium sulfate fed to growing-finishing steers on d 0 and 7 simulated retail display steak subcutaneous fat fatty acid (FA) composition.

	Treatment[1]							
	d 0		d 7			*P*-value[2]		
FA composition,[3] %	LS	HS	LS	HS	SEM	Trt	Day	Trt × Day
No. of samples[4]	9	10	2	4				
SFA								
Total[5]	46.06	46.03	47.57	48.58	1.219	0.73	0.14	0.68
Capric (10:0)	0.031	0.038	0.017	0.014	0.008	0.83	0.06	0.55
Lauric (12:0)	0.060	0.064	0.054	0.060	0.005	0.48	0.14	0.73
Myristic (14:0)	3.32	3.38	3.13	3.37	0.196	0.52	0.61	0.62
Pentadecanoic (15:0)	0.52	0.56	0.48	0.52	0.028	0.29	0.17	0.99
Palmitic (16:0)	27.52	26.87	27.47	27.25	0.469	0.51	0.42	0.29
Margaric (17:0)	1.26	1.26	1.36	1.33	0.049	0.77	0.11	0.73
Stearic (18:0)	14.43	14.88	16.65	17.53	0.771	0.45	0.02	0.80
Arachidic (20:0)	0.094	0.103	0.105	0.113	0.006	0.21	0.15	0.96

Table 7 (Cont.)

FA composition,[3] %	Treatment[1] d 0 LS	d 0 HS	d 7 LS	d 7 HS	SEM	*P*-value[2] Trt	Day	Trt × Day
MUFA								
Total[6]	47.44	47.18	47.21	45.68	1.304	0.54	0.53	0.64
Myristoleic (14:1)	0.98	1.06	0.73	0.91	0.085	0.22	0.04	0.52
Palmitelaidic (16:1*trans*)	0.35	0.39	0.38	0.37	0.017	0.55	0.91	0.22
Palmitoleic (16:1*cis*)	3.46	3.22	3.61	3.34	0.134	0.15	0.14	0.88
Heptadecenoic (17:1*trans*)	0.15	0.15	0.15	0.15	0.006	0.95	0.55	0.63
Total 18:1*trans* FA	2.51	2.66	2.55	2.67	0.156	0.51	0.78	0.88
Oleic (18:1*cis*9)	38.49	38.03	39.43	37.78	1.135	0.41	0.77	0.61
Vaccenic (18:1*trans*11)	0.98	0.68	0.66	0.64	0.207	0.47	0.41	0.52
Gadoleic (20:1*cis*11)	0.22	0.21	0.20	0.23	0.028	0.79	0.84	0.46
PUFA								
Total[7]	2.40	2.42	2.33	2.30	0.102	0.97	0.17	0.72
Linoleic (18:2*n*-6)	1.33	1.26	1.35	1.27	0.056	0.35	0.68	0.87

Table 7 (Cont.)

	Treatment[1]							
	d 0		d 7			*P*-value[2]		
FA composition,[3] %	LS	HS	LS	HS	SEM	Trt	Day	Trt × Day
Total CLA[8]	0.66	0.73	0.61	0.64	0.046	0.39	0.09	0.59
18:2*cis*9*trans*11	0.60	0.67	0.54	0.56	0.042	0.38	0.05	0.48
18:2*trans*10*cis*12	ND[9]	ND	ND	ND				
18:2*cis*9*cis*11	0.02	0.02	0.03	0.03	0.027	0.94	0.11	0.56
18:2*trans*9*trans*11	0.04	0.05	0.04	0.04	0.007	0.77	0.24	0.91
α-Linolenic (18:3*n*-3)	0.24	0.27	0.23	0.25	0.017	0. 27	0.26	0.47
γ-Linolenic (18:3*n*-6)	0.003	0.003	0.004	0.003	0.002	0.77	0.63	0.18
Eicosadienoic (20:2)	0.020	0.018	0.020	0.019	0.003	0.63	0.72	0.45
Eicosatrienoic (20:3*n*-3)	0.007	0.010	0.008	0.008	0.002	0.52	0.77	0.36
Dihomo-γ-linoleic (20:3*n*-6)	0.063	0.054	0.059	0.050	0.003	0.04	0.22	0.90
Arachidonic (20:4*n*-6)	0.039	0.030	0.030	0.025	0.004	0.20	0.06	0.41
Eicosapentaenoic (20:5*n*-3)	0.006	0.008	0.008	0.008	0.002	0.91	0.71	0.68
Docosapentaenoic (22:5*n*-3)	0.040	0.030	0.028	0.024	0.007	0.47	0.17	0.64

Table 7 (Cont.)

FA composition,[3] %	Treatment[1] d 0 LS	d 0 HS	d 7 LS	d 7 HS	SEM	*P*-value[2] Trt	Day	Trt × Day
Docosahexaenoic (22:6*n*-3)	ND	ND	ND	ND				
Total unsaturated FA[10]	49.83	49.02	49.76	47.90	1.232	0.33	0.64	0.69
Total *trans* FA[11]	3.00	3.20	3.06	3.19	0.171	0.47	0.83	0.77
Total *n*-6 FA[12]	1.43	1.35	1.43	1.35	0.062	0.33	0.95	0.93
Total *n*-3 FA[13]		0.32	0.27	0.29	0.022	0.39	0.05	0.69
n-6:*n*-3[14]	5.16	4.26	5.39	4.69	0.378	0.14	0.11	0.56
PUFA:SFA[15]	0.052	0.053	0.050	0.048	0.003	0.80	0.18	0.58
Unsaturated FA:SFA[16]	1.08	1.05	1.06	0.98	0.048	0.34	0.34	0.69

[1]LS = low S and HS = high S.
[2]*P*-values for the effect of dietary treatment (Trt), day (Day), and their interaction (Trt × Day).
[3]Amount of FA in sample expressed on a weight percentage basis as determined by gas chromatography.
[4]Fatty acid composition unavailable for multiple d 7 samples owing to a freeze dryer malfunction. Samples were subjected to transesterification in duplicate.
[5]Total saturated fatty acids (SFA) = 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0.
[6]Total monounsaturated fatty acids (MUFA) = 14:1, 16:1*trans*, 16:1*cis*, 17:1*trans*, total 18:1*trans* FA, 18:1*cis*9, 18:1*cis*11, and 20:1*cis*11.
[7]Total polyunsaturated fatty acids (PUFA) = 18:2*n*-6, total CLA, 18:3*n*-3, 18:3*n*-6, 20:2, 20:3*n*-3, 20:3*n*-6, 20:4*n*-6, 20:5*n*-3, 22:5*n*-3, and 22:6*n*-3.

Table 7 (Cont.)

[8]Total conjugated linoleic acid (CLA) = 18:2*cis*9*trans*11, 18:2*trans*10*cis*12, 18:2*cis*9*cis*11, and 18:2*trans*9*trans*11.

[9]ND = not detected.

[10]Total unsaturated FA = total MUFA and total PUFA.

[11]Total *trans* FA = 16:1*trans*, 17:1*trans*, and total 18:1*trans* FA

[12]Total *n*-6 FA = 18:2*n*-6, 18:3*n*-6, 20:3*n*-6, and 20:4*n*-6.

[13]Total *n*-3 FA = 18:3*n*-3, 20:3*n*-3, 20:5*n*-3, 22:5*n*-3, and 22:6*n*-3.

[14]*n*-6:*n*-3 = total *n*-6 FA/total *n*-3 FA.

[15]PUFA:SFA = total PUFA/total SFA.

[16]Unsaturated FA:SFA = total unsaturated FA/total SFA.

CHAPTER IV

Excess dietary sulfur from sodium sulfate in beef steer growing-finishing diets: Effects on steer mineral status, sulfhemoglobin concentrations, and mitochondria cytochrome *c* oxidase activity

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ABSTRACT

The objective of this study was to determine the effects of excess dietary S using sodium sulfate (**Na_2SO_4**) on mineral status, sulfhemoglobin (**SHb**) concentrations, and mitochondria cytochrome *c* oxidase activity in growing-finishing beef cattle. Twenty steers of predominantly Angus breeding were stratified by body weight (**BW**; 279 ± 13.2 kg), assigned randomly to 6, 2.4-ha paddocks (3 to 4 steers/paddock), and supplemented with either low S ground corn and soybean meal supplement that did not contain any byproduct feeds (0.31% total dietary S; **LS**) or LS supplement with an additional 0.25% S provided from Na_2SO_4 (0.58% total dietary S; **HS**) for 114 d. Steers were moved to feedlot (BW = 373 ± 0.2 kg), remained on prior dietary S treatments, and were fed corn and soybean meal diets (0.19 and 0.42% total dietary S; LS and HS treatments, respectively) that did not contain any byproduct feeds with no use of growth-enhancing technologies for 123 d. Steers were harvested (BW = 564 ± 7.7 kg) in a commercial abattoir. Total plasma Cu and Zn concentrations were not affected ($P \geq 0.74$) by dietary treatments during the growing phase. During the finishing phase, plasma Cu concentrations were less ($P = 0.07$) in steers fed HS than steers fed LS, whereas plasma Zn concentrations were

greater ($P = 0.02$) in steers fed HS; however, plasma Cu concentrations were within the normal range and did not approach concentrations indicative of deficiency. Steers fed HS had greater ($P \leq 0.09$) liver Ca and K concentrations at slaughter than those fed LS. Remaining liver minerals were not affected ($P \geq 0.21$) by treatment and were within the normal range established for growing cattle. Longissimus muscle mineral concentrations at slaughter were not affected ($P \geq 0.33$) by dietary treatment. Sulfhemoglobin production was greater ($P < 0.001$) in steers fed HS when compared to steers fed LS (0.45 and 0.37%, respectively), but this increase was likely insufficient to cause hypoxia, with the greatest SHb concentration (0.61% of total hemoglobin) observed in steers fed HS on d 234. Cytochrome *c* oxidase activity in liver and LM tissues was not affected ($P \geq 0.38$) by dietary treatment. These data suggest exposing growing-finishing beef cattle to greater S diets via supplemental dietary S from Na_2SO_4 influences plasma mineral status, does not impact on LM mineral concentrations and cytochrome *c* oxidase activity, and has limited impact on liver mineral concentrations.

Key words: beef cattle, mineral status, cytochrome *c* oxidase activity, sodium sulfate, sulfhemoglobin

INTRODUCTION

Increased use of corn distillers grains in ruminant diets has led to considerable research of the effects of high dietary S. Studies evaluating dietary S level in cattle diets have used acidic (e.g., sulfuric acid, various types of corn distillers grains, elemental S, or ammonium sulfate), neutral [e.g., sodium sulfate (**Na_2SO_4**)], basic (e.g., calcium sulfate), or a mixture of dietary S sources.

Ruminants fed excess dietary S from acidic sources have been reported to experience decreased apparent Cu absorption (Arthington et al., 2002; Spears et al., 2011; Felix and Loerch,

2011; Felix et al., 2012; Pogge and Hansen, 2013; Ponce et al., 2014), increased apparent S absorption (Felix and Loerch, 2011; Salim et al., 2012; Ponce et al., 2014), and increased blood sulfhemoglobin (**SHb**) concentrations (Pogge and Hansen, 2013), whereas cytochrome *c* oxidase (**CytOx**) activity was unaffected (Ponce et al., 2014). However, when using neutral dietary S sources or adding neutral S sources to increase the S concentration of corn distillers grains based diets to test hypotheses on dietary S level, results may not generalize to acidic based diets. Felix et al. (2014) reported that dietary S source impacted ruminal S metabolism, as S from dried corn distillers grains with solubles (**DDGS**) or a corn-based diet with sulfuric acid increased ruminal H_2S concentrations when compared to a neutral dietary S source (i.e., Na_2SO_4).

As ruminants may adapt to and metabolize dietary S sources differently, there is a need to understand if the mineral status and biomarkers associated with high concentrations of dietary S differ when Na_2SO_4 is fed. The objective of this study was to evaluate the effects of excess dietary S using supplemental Na_2SO_4 on growing-finishing steer mineral status, blood SHb concentrations, and CytOx activity. It was hypothesized that an increase in systemic S from dietary Na_2SO_4 would increase S accumulation in the liver and muscle and blood SHb concentrations with a concomitant decrease in liver Cu accumulation and liver and longissimus muscle (**LM**) CytOx activity.

MATERIALS AND METHODS

Animal handling procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (protocol #13008) and followed guidelines recommended in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010). The study was conducted at the University of Arkansas Stocker Cattle Receiving and Backgrounding Unit (Savoy, AR).

Animals and experimental design

Twenty steers [initial body weight (**BW**) = 279 ± 13.2 kg] of predominantly Angus breeding were obtained from the University of Arkansas Cow-Calf Unit (Savoy, AR). Steers were stratified by initial BW and assigned randomly to 1 of 6 paddocks (3 to 4 steers/paddock) for a 114-d growing phase. Paddocks were assigned randomly to 1 of 2 dietary treatments: 1) low S ground corn and soybean meal supplement (**LS**) or 2) LS supplement with an additional 0.25% S provided from Na_2SO_4 (**HS**; Table 1). Steers grazed mixed grass [0.31% S, 17.8% crude protein, 26.8% acid detergent fiber, 57.1% neutral detergent fiber, and 10.4% ash, dry matter (**DM**) basis] paddocks (2.4-ha/paddocks) and were supplemented to meet NRC (2000) nutrient requirements for 0.15% S with a ground corn and soybean meal diet that did not contain any byproduct feeds (LS treatment). Remaining steers were offered an identical supplement to which an additional 0.25% S from Na_2SO_4 (Prince Agri Products, Inc., Quincy, IL) had been added in the total diet DM. Steers did not receive implants. In the event forage became limiting, steers were provided access to bermudagrass hay (0.36% S, 18.8% crude protein, 33.0% acid detergent fiber, 70.0% neutral detergent fiber, and 10.3% ash, DM basis) in quantities sufficient to ensure ad libitum access to forage. When the average BW of the steers reached 373 ± 0.2 kg, steers were stratified within dietary treatment by BW and assigned randomly to 16 dry-lot pens (1 to 2 steers/pen; 8 pens/dietary treatment). Steers remained on the same dietary treatment for a 123-d finishing phase. Steers on the LS treatment were offered a traditional corn and soybean meal finishing diet that did not contain any byproduct feeds or ionophore supplementation and met the NRC (2000) requirement of 0.15% S, whereas steers on the HS treatment steers were offered an identical diet except an additional 0.25% S was provided from Na_2SO_4 (Table 1).

Sample collection and analytical procedures

Mineral status. Jugular blood was collected in sodium heparin tubes (BD Vacutainer; Becton Dickinson Co., Franklin Lakes, NJ) for plasma trace mineral analysis from all steers on d 0, 28, 56, 84, 112, 140, 168, 196, and 234. Blood was transported to the laboratory on ice and centrifuged (1,200 × *g*, 20 min at 20°C). Plasma was aliquoted and stored at -80°C until trace mineral analysis. Plasma was prepared for analysis with a 1:9 (vol/vol) dilution of plasma in 1 *N* trace mineral-grade nitric acid. Samples were vortexed vigorously to precipitate protein and centrifuged (1,200 × *g*, 20 min at 20°C). Supernatant was collected and analyzed for Cu and Zn content by inductively coupled plasma atomic emission spectroscopy at a commercial laboratory (method 975.03; AOAC, 1988; Altheimer Laboratory, Fayetteville, AR). Instrument accuracy for mineral analyses was verified using bovine liver standard (1577c; National Institute of Standards and Technology, Gaithersburg, MD).

Liver and LM tissue samples for trace mineral analysis were collected from all steers postmortem after slaughter. Steers were harvested on the same date when the 12th-rib fat thickness was estimated to have reached 1-cm. Feed was withheld overnight with free access to water, and steers were transported 351 km for harvest in a commercial abattoir (Creekstone Farms, Arkansas City, KS). Longissimus muscle samples were taken from all steers between the 12th and 13th-rib section. All tissue samples were snap frozen in liquid nitrogen and transported back to the lab and stored at -80°C until trace mineral analysis. Liver and LM samples were dried in a forced-air oven at 50°C for approximately 1 wk until dried completely. Dried liver and LM samples were analyzed for complete minerals as previously described for plasma mineral analysis.

Sulfhemoglobin. Jugular blood was collected in K_2-EDTA tubes (BD Vacutainer; Becton Dickinson Co.) from all steers prior to feeding on d 0, 28, 56, 84, 112, 140, 168, 196, and 234 for total hemoglobin (**Hb**) and SHb analysis in accordance to the procedure described by Evelyn and Malloy (1938). Sulfhemoglobin concentration was calculated using the extinction coefficient (Carrico et al., 1978). Reference absorbance was established using assay buffer. Samples were analyzed in triplicate and internal controls were used to determine inter- and intra-assay CV (6.4 and 2.5%, respectively). Total Hb and SHb concentrations were determined within 8 h of sample collection.

Mitochondria isolation and CytOx activity. Liver and LM samples for mitochondria isolation were collected from all steers postmortem after slaughter and stored until analysis as previously described. Liver mitochondria isolation (Fig. 2) was adapted from Graham (1999) and Pallotti and Lenaz (2007), whereas LM mitochondria isolation (Fig. 3) was adapted from Bhattacharya et al. (1991) and Graham (1999).

Mitochondria isolation consists of 4 steps: 1) cells are swollen in a hypotonic buffer; 2) cells are ruptured with a Dounce or Potter-Elvehjem homogenizer using a tight-fitting pestle; 3) differential centrifugation (first at low speed to pellet mainly nuclei and unbroken cells and then at high speed to pellet mitochondria); and 4) washing the mitochondrial pellet to reduce the presence of subcellular contaminants. The washing steps are critical for increasing mitochondria purity. Suspending the pellet is achieved by pipetting. All solutions, glassware, centrifuge tubes, and equipment required for mitochondria isolation are precooled to 0 to 4°C and kept on ice throughout to minimize protease and phospholipase activation. Thermally insulated gloves are worn to mitigate skin heat transfer. Mitochondria from different sources are obtained using basically the same methodology, although modifications may be introduced depending on tissue

type. Liver contains a considerable amount of mitochondria and is easier to manipulate than is skeletal muscle.

Frozen liver tissue, on average 0.61 g, range 0.47 to 0.81 g, was thawed (0.25 *M* sucrose, 0.01 *M* Tris-HCl, pH 7.5) at 45°C, weighed, rinsed with liver homogenization medium (**LHM**; 0.2 *M* mannitol, 50 m*M* sucrose, 10 m*M* KCl, 1 m*M* Na_2EDTA, 10 m*M* HEPES, pH 7.4 with KOH), diced into small pieces using scissors, and incubated for 5 min on ice for hypotonic cell swelling (Fig. 2). Liver pieces were homogenized in LHM to rupture cells with 5 strokes of a Potter-Elvehjem homogenizer (Potter S; Sartorius North America Inc., Edgewood, NY) fitted with a Teflon pestle at a speed of 500 rpm. After incubation, the homogenate was collected and centrifuged (1,000 × *g*, 10 min at 4°C). Supernatant was collected and centrifuged (3,000 × *g*, 10 min at 4°C). Using a glass Pasteur pipet attached to a vacuum pump (VacuMaster Single-stage Vacuum Pump; Robinair, Warren, MI), the supernatant fraction was aspirated and discarded, and the resulting pellet washed with LHM and centrifuged as before 3 times. The final pellet, containing the mitochondria, was stored in 10.0 μL aliquots at -80°C as a concentrated suspension in LHM.

Frozen LM, on average 1.24 g, range 0.88 to 1.75 g, was thawed as previously described for liver mitochondria isolation, weighed, rinsed twice with muscle wash buffer (0.2 *M* mannitol, 70 m*M* sucrose, 0.1 m*M* Na_2EDTA, 10 m*M* Tris-HCl, pH 7.4), and trimmed of fat and connective tissue (Fig. 3). Rinsed tissue was diced into small pieces using scissors and incubated for 5 min on ice in muscle homogenization medium 1 (**MHM_1**; 0.1 *M* sucrose, 46 m*M* KCl, 10 m*M* Na_2EDTA, 10 m*M* Tris-HCl, pH 7.4, 0.5% BSA, 0.2 mg/mL Nargase) for hypotonic cell swelling. Muscle pieces were homogenized to rupture cells in MHM_1 with 8 strokes of a Potter-Elvehjem homogenizer (Potter S; Sartorius North America Inc.) fitted with a Teflon pestle at a

speed of 700 rpm and incubated on ice for 5 min. The use of a commercially available protease to facilitate LM homogenization reduced the severity of the shear forces needed to disrupt the tissue, therefore minimizing damage to the mitochondria. Homogenate was diluted with muscle homogenization medium 2 (**MHM_2**; 0.1 *M* sucrose, 46 m*M* KCl, 10 m*M* Na_2EDTA, 10 m*M* Tris-HCl, pH 7.4) and homogenized as before. After incubation, the homogenate was collected and centrifuged (2,000 × *g*, 10 min at 4°C). Supernatant was collected and centrifuged (10,000 × *g*, 10 min at 4°C). The supernatant fraction was aspirated and discarded as previously described for liver mitochondria isolation, and the resulting pellet washed with MHM_2 and centrifuged as before. The final pellet was stored in 10.0 μL aliquots at -80°C as a concentrated suspension in MHM_2.

Mitochondria protein concentration was estimated using a standard protein assay kit (Coomassie Protein Assay Kit; Pierce Biotechnology, Rockford, IL) to allow standardization of results to a constant tissue protein concentration. Reference absorbance was established using respective sample diluents. Samples were analyzed in triplicate and internal controls were used to determine inter- and intra-assay CV (4.8 and 2.6%, respectively). Cytochrome *c* oxidase activity was determined by measuring the oxidation rate of reduced cytochrome *c* using a colorimetric assay kit (MitoCheck Complex IV Activity Assay Kit; Cayman Chemical, Ann Arbor, MI). The spectrophotometer (SpectraMax M5; Molecular Devices, San Jose, CA) was set to monitor the decrease in absorbance at 550 nm at 25°C every 30 s for 15 min using a kinetic program. Reference absorbance was established using assay buffer. All reagents were prepared the same day of analysis and handled as specified by the manufacturer. Samples were analyzed in duplicate and internal controls were used to determine inter- and intra-assay CV (3.0 and 2.2%, respectively).

Statistical analysis

Data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Inst., Inc., Cary, NC) by means of a compound symmetry covariance structure. Denominator degrees of freedom were estimated using the Kenward-Rogers option. Data adherence to the assumptions of the statistical test was established. Liver and LM mineral, mitochondria protein, and CytOx activity data were analyzed as randomized designs. Dietary treatment was the fixed effect and pen (experimental unit) was included as the random effect in the models. Plasma mineral and SHb data were analyzed as repeated measures. Fixed effects included in the model were dietary treatment and day, as well as the 2-way interaction, whereas pen (experimental unit) was included as a random effect. Day was included as a repeated effect. Least squares means were partitioned at the 10% level of significance by way of the probability of differences option. Statistical significance was declared at $P \leq 0.10$.

RESULTS AND DISCUSSION

The objective of this study was to understand the effects of excess dietary S using supplemental Na_2SO_4 on growing-finishing steer mineral status, blood SHb concentrations, and CytOx activity. It was hypothesized that an increase in systemic S from dietary Na_2SO_4 would increase S accumulation in the liver and muscle and blood SHb concentrations with a concomitant decrease in liver Cu accumulation and liver and LM CytOx activity. These data suggest exposing growing-finishing cattle to greater S diets via supplemental dietary S from Na_2SO_4 influences plasma mineral status, did not impact LM mineral concentrations and CytOx activity, and had limited impact on liver mineral concentrations. However, when interpreting the results of this study, there are a couple factors that should be considered. Greater steer numbers would increase the power for detection of differences in liver and LM mineral concentrations and

CytOx activity. In light of the small sample size per treatment group, the power to detect significant differences was limited and a factor in the choice to use a type I error rate of 0.10. Moreover, the lack of studies that used Na_2SO_4 for comparison prohibited establishing a substantiated relationship between treatment and outcome.

Mineral status

Plasma Cu and Zn concentrations were not affected ($P \geq 0.74$) by dietary treatments during the growing phase (Table 2); however, an overall increase ($P < 0.0001$) in plasma Cu and Zn concentrations was observed for both treatments. During the finishing phase, plasma Cu concentrations were less ($P < 0.10$) in steers fed HS than steers fed LS, whereas plasma Zn concentrations were greater ($P = 0.02$) in steers fed HS. The decreased plasma Cu and concomitant increased plasma Zn concentrations suggests absorption through similar pathways and competition for absorption sites (Oestreicher and Cousins, 1985). Moreover, decreased plasma Cu may also be attributed to an antagonistic interaction between Cu and S. Sulfur in the form of sulfide ions is believed to reduce Cu bioavailability by forming insoluble and biologically unavailable CuS in the rumen (Suttle, 1974). Total dietary S for the finishing phase HS treatment exceeded the 0.30% of dietary DM suggested by NRC (2005) as the maximum tolerable concentration for high concentrate diets. However, plasma Cu concentrations were within the normal range and did not approach concentrations indicative of deficiency (0.2 to 0.5 mg/L; Kincaid, 1999) due to sufficient dietary Cu in the relative absence of other Cu antagonists (e.g., Fe).

Steers fed HS had greater ($P \leq 0.09$) liver Ca and K concentrations at slaughter than those fed LS (Table 3). Remaining liver minerals were not affected ($P \geq 0.21$) by treatment and within the normal range established for growing cattle (Kincaid, 1999; Underwood and Suttle, 1999).

Longissimus muscle mineral concentrations at slaughter were not affected ($P \geq 0.33$) by dietary treatment (Table 4). It was hypothesized that an increase in systemic S would cause increased S accumulation in the liver and muscle, but this was not the case. The lack of a detectable change in the S concentration of tissues from steers fed HS suggests that increased S absorption did not result in increased S deposition. Instead, S homeostasis may have been regulated at the renal level. Weeth and Capps (1972) observed increasing the total dietary S from 0.20 to 0.63% by supplementing drinking water with Na_2SO_4 [110, 1,462, and 2,814 mg sulfate ($\mathbf{SO_4^{2-}}$)/L; 0.20, 0.41, and 0.63% total (water plus feed) dietary S, respectively] increased urine SO_4^{2-} in growing heifers. Salim et al. (2012) compared DDGS in corn based finishing diets at 0, 16.7, 33.3, and 50% of diet DM (0.13, 0.23, 0.33 and 0.45% S, respectively). Urinary S excretion linearly increased with increasing DDGS concentration. Felix et al. (2012) observed S accumulation in the kidneys and total urine output linearly increase with increasing levels of DDGS (0, 20, 40, or 60% DM; 0.13, 0.18, 0.29, or 0.35% S, respectively) replacing ground corn. Therefore, in the current study, excess absorbed S may have been excreted via the urine to prohibit increased S deposition in the liver and muscle.

Contrary to the hypothesis, an increase in systemic S from Na_2SO_4 did not decrease Cu accumulation in the liver. Liver Cu may have been unaffected by the inclusion of Na_2SO_4 in the diet as the concentration of Cu in the HS diet (18 mg/kg) was adequate for liver Cu depletion not to occur. Cammack et al. (2010) observed a reduction in plasma and liver Cu concentrations when administering drinking water supplemented with Na_2SO_4 [566 and 3,651 mg SO_4^{2-}/L; 0.28 and 0.79% total (water plus feed) dietary S, respectively] to forage-fed steers. However, the authors attributed the disruption of Cu absorption to the formation Cu-S-Mo complexes from the high dietary S the moderate amount dietary Mo present in the diets. Dietary S source acidity may

also play an important role in liver Cu concentrations. Sodium sulfate is a neutral dietary S source, but recent studies evaluating the effects of dietary S level in ruminant diets have mainly used acidic [e.g., various types of corn distillers grains or ammonium sulfate] dietary S sources. In contrast to the results of the current study, decreased liver Cu concentrations have been reported with increasing quantities of dietary S from acidic dietary S sources (Arthington et al., 2002; Spears et al., 2011; Felix et al., 2012; Richter et al., 2012; Ponce et al., 2014). Increasing the S concentration in grasses from 0.22 to 0.51% with ammonium sulfate fertilizer (Arthington et al., 2002) decreased liver Cu accumulation. In the previously mentioned study by Felix et al. (2012), liver Cu accumulation linearly decreased with increasing levels of DDGS when replacing ground corn in feedlot lamb diets. Similarly, Ponce et al. (2014) observed increasing dietary S from 0.30 to 0.60% decreased Cu accumulation in the liver when replacing steam-flaking corn with wet corn distillers grains with solubles (**WDGS**; 0, 30, or 60% DM; 0.14, 0.26, or 0.41% S, respectively). These results suggest that the chemical form of dietary S administered may influence liver Cu.

Sulfhemoglobin

Total Hb and SHb concentrations suggest that steers fed excess dietary S from supplemental Na_2SO_4 influences abnormal blood derivative formation. A treatment × day interaction ($P = 0.05$) was detected for total Hb during the finishing phase, as steers fed HS exhibited greater ($P = 0.03$) total Hb concentrations on d 196 when compared to steers fed LS (Table 2). During the growing phase, greater SHb concentrations were observed on d 28, 56, 84, and 112 (treatment × day interaction, $P < 0.001$) in steers fed HS than steers fed LS, whereas SHb was greater ($P < 0.001$) throughout the finishing phase in steers fed HS when compared to steers fed LS. Sulfhemoglobin production increased ($P < 0.01$) over time during the growing and

finishing phases for both treatments. Dietary S present in the rumen contributes to the production of sulfide ions. Sulfide ions can be absorbed across the rumen wall into the portal blood to oxidize the heme moiety in functional Hb to form irreversible SHb, making the Hb incapable of carrying oxygen and leading to hypoxia (Drabkin and Austin, 1935; Bulgin et al., 1996). In the current study, SHb concentrations were greater ($P < 0.001$) in steers fed HS when compared to steers fed LS (0.45 and 0.37%, respectively). However, this increase was likely insufficient to cause hypoxia, with the greatest SHb concentration (0.61% of total Hb) observed in steers fed HS on d 234. Physiologic anemia was anticipated to occur in steers fed HS due to the inability of Hb to carry oxygen to tissues, but total Hb fell within the normal reference range for growing steers (Doornenbal et al., 1988). Silver et al. (1956) induced sulfhemoglobinemia in rabbits and dogs and reported increased hemolysis accompanied by a compensatory hematopoiesis similar to that observed in the current study. The mechanism by which steers fed HS stimulated erythropoietic activity to maintain total Hb is likely due to the action of erythropoietin. Erythropoietin is a glycoprotein hormone produced in the kidney that acts on erythroid progenitor cells in the bone marrow to maintain red blood cell mass following an anaemic or hypoxaemic stimulus (Jelkmann, 2011). Therefore, the negative feedback system, in which tissue oxygenation controls erythropoietin production and erythropoietin controls red blood cell production, likely provided steers fed HS total Hb homeostasis.

Reported effects of increased dietary S from Na_2SO_4 on abnormal blood derivative formation in growing cattle are variable. Consistent with the findings in the current study, Weeth and Capps (1972) observed increasing the total dietary S from 0.20 to 0.63% by supplementing drinking water with Na_2SO_4 [110, 1,462, and 2,814 mg SO_4^{2-}/L; 0.20, 0.41, and 0.63% total (water plus feed) dietary S, respectively] increased the concentration of SHb in growing heifers.

Similarly, Drewnoski et al. (2012) observed increased SHb production in growing steers when increasing the total dietary S from 0.24 to 0.68% with Na_2SO_4. Conversely, previous research demonstrated increasing the total dietary S intake of growing cattle with supplemental Na_2SO_4 did not impact SHb concentrations (Digesti and Weeth, 1976; Gould et al., 1997). Digesti and Weeth (1976) administered drinking water supplemented with Na_2SO_4 [110, 1,250, and 2,500 mg SO_4^{2-}/L; 0.20, 36, and 0.57% total (water plus feed) dietary S, respectively] to growing heifers for 90 d. Increasing total dietary S did not impact SHb concentrations, but heifers drinking sulfate-water exhibited increased methemoglobin production with the increase in the 1,250 mg SO_4^{2-}/L treatment being significant. Cattle readily oxidize Hb to methemoglobin (Smith and Gosselin, 1964), and methemoglobin formation by sodium nitrite has been reported to provide prophylaxis against toxic sulfide concentrations (Smith, 1969). Accordingly, SHb formation may have been prevented due to the conversion of functional Hb to methemoglobin rather than the formation of SHb. Gould et al. (1997) observed that SHb concentrations did not increase when feeding steers a diet high in readily fermentable carbohydrate and low in long fiber with 1.8% Na_2SO_4 for 17 d. These results were attributed to an insufficient exposure time to high-sulfate diets for SHb levels to increase.

Cytochrome c oxidase activity

It was hypothesized that an increase in systemic S would decrease CytOx activity in the tissues from steers fed HS; however, CytOx activity in liver and LM tissues was not affected ($P \geq 0.38$) by dietary treatment (Table 5). In ruminants, the formation of hydrogen sulfide (**H_2S**) is a function of the sulfide concentration and rumen pH (Bulgin et al., 1996), with decreased ruminal pH causing H_2S to be released into the gas cap as it is insoluble in water. Hydrogen sulfide present in the rumen gas cap readily diffuses into the portal blood stream where it readily

dissociates due to the increased pH of blood (Niles et al., 2002). Sulfide ions and H_2S inhibit CytOx activity in the electron transport system to decrease oxidative metabolism in mitochondria (Pandher, 2000). The absence of a detectable change in CytOx activity from the tissues of steers fed HS suggests that Na_2SO_4 supplementation inadequately decreased ruminal pH to liberate insufficient H_2S to adversely affect oxidative metabolism.

In the previously mentioned study by Ponce et al. (2014), cattle fed a 60% WDGS diet experienced greater H_2S concentrations in the rumen gas cap when compared to steers fed a 30% WDGS diet, but liver and muscle CytOx activity were consistent between treatments. Interestingly, brain tissue from cattle fed 60% WDGS tended to have lower CytOx activities than brain tissue from cattle fed 30% WDGS. Sulfite is generated in ruminants during the process of sulfide elimination. Sulfite is a potent nucleophile capable of causing considerable oxidative damage, and the brain tissue, with its large lipid content, is very susceptible to oxidative damage (Pandher, 2000). The tendency for decreased CytOx activity in brain tissue from cattle fed 60% WDGS in Ponce et al. (2014) and proposed pathophysiologic mechanism of S toxicosis suggests that brain tissue collections may have been warranted in the current study to evaluate the effects of excess dietary S using supplemental Na_2SO_4 on CytOx activities.

The results of the present study provide insight into the mineral status and biomarkers associated with high concentrations of dietary S differ when Na_2SO_4 is fed to growing-finishing steers. Exposing growing-finishing cattle to greater S diets via supplemental dietary S from Na_2SO_4 decreased plasma Cu concentrations in steers during the finishing phase, but plasma Cu concentrations were within the normal range and did not approach concentrations indicative of deficiency. The effect of feeding Na_2SO_4 was also characterized by increased blood SHb concentrations, though the increase was unlikely to cause hypoxia. The absence of a detectable

change in CytOx activity in liver and LM from steers fed HS suggests dietary Na_2SO_4 may not influence oxidative metabolism. However, the effects of excess dietary S from Na_2SO_4 on CytOx activity inhibition warrant further consideration using brain tissue due to the pathophysiologic mechanism of S toxicosis.

ACKNOWLEDGMENTS

This research was supported by the Arkansas Beef Council. The authors thank J. Reynolds and D. Galloway (University of Arkansas, Department of Animal Science, Fayetteville, AR) for assistance with tissue specimen collection.

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Table 1. Ingredient and analyzed composition of corn and soybean meal diets fed to steers.

	Treatment			
	Growing phase[1,2]		Finishing phase[3]	
Item	LS	HS	LS	HS
Ingredient, % as-fed basis				
Corn grain, cracked	81.2	80.4	76.3	76.1
Bermudagrass hay	--	--	7.0	7.0
Soybean meal	12.6	12.6	6.0	6.0
Cottonseed hulls	--	--	5.0	5.0
Molasses, cane	1.8	1.8	3.0	3.0
Calcium carbonate	0.45	0.45	1.0	1.0
Salt	3.30	0.00	0.85	0.00
Sodium sulfate, anhydrous[4]	0.00	4.10	0.00	1.05
Urea	--	--	0.80	0.80
Trace mineral premix[5]	0.10	0.10	0.025	0.025
Vitamin A, D, and E[6]	0.10	0.10	0.05	0.05
Rumensin premix[7]	0.40	0.40	--	--
Analyzed composition,[8] DM basis				
CP, %	14.9	14.5	14.3	15.1
ADF, %	2.1	2.0	6.4	6.6
NDF, %	16.5	17.2	23.7	27.7
Ca, %	0.48	0.38	0.68	0.66
P, %	0.43	0.40	0.34	0.36

Table 1 (Cont.)

	Treatment			
	Growing phase[1,2]		Finishing phase[3]	
Item	LS	HS	LS	HS
S, %	0.30	1.31	0.19	0.42
Na, %	1.96	1.73	0.43	0.37
Cu, mg/kg	73	60	24	18
Fe, mg/kg	203	177	113	99
Mn, mg/kg	166	132	71	56
Zn, mg/kg	270	239	108	85

[1]Fed at a rate of 1.8 $kg \cdot d^{-1} \cdot hd^{-1}$ to cattle grazing mixed grass pasture and also offered ad libitum access to bermudagrass hay.
[2]LS = low S, 0.31% total dietary S and HS = high S, 0.58% total dietary S.
[3]LS = low S, 0.19% total dietary S and HS = high S, 0.42% total dietary S.
[4]Prince Agri Products, Inc., Quincy, IL.
[5]Trace mineral premix composition (mg/kg): 500 Co; 40,000 Cu; 2,000 I; 10,000 Fe; 80,000 Mn; 600 Se; and 120,000 Zn (Nutrablend, Neosho, MO).
[6]Vitamin premix composition (IU/kg): 1,816,000 vitamin A; 36,320 vitamin D; and 227 vitamin E.
[7]Rumensin 80 (Elanco, Indianapolis, IN) supplied 22 mg of monensin/kg of diet DM.
[8]CP = crude protein; ADF = acid detergent fiber; and NDF = neutral detergent fiber.

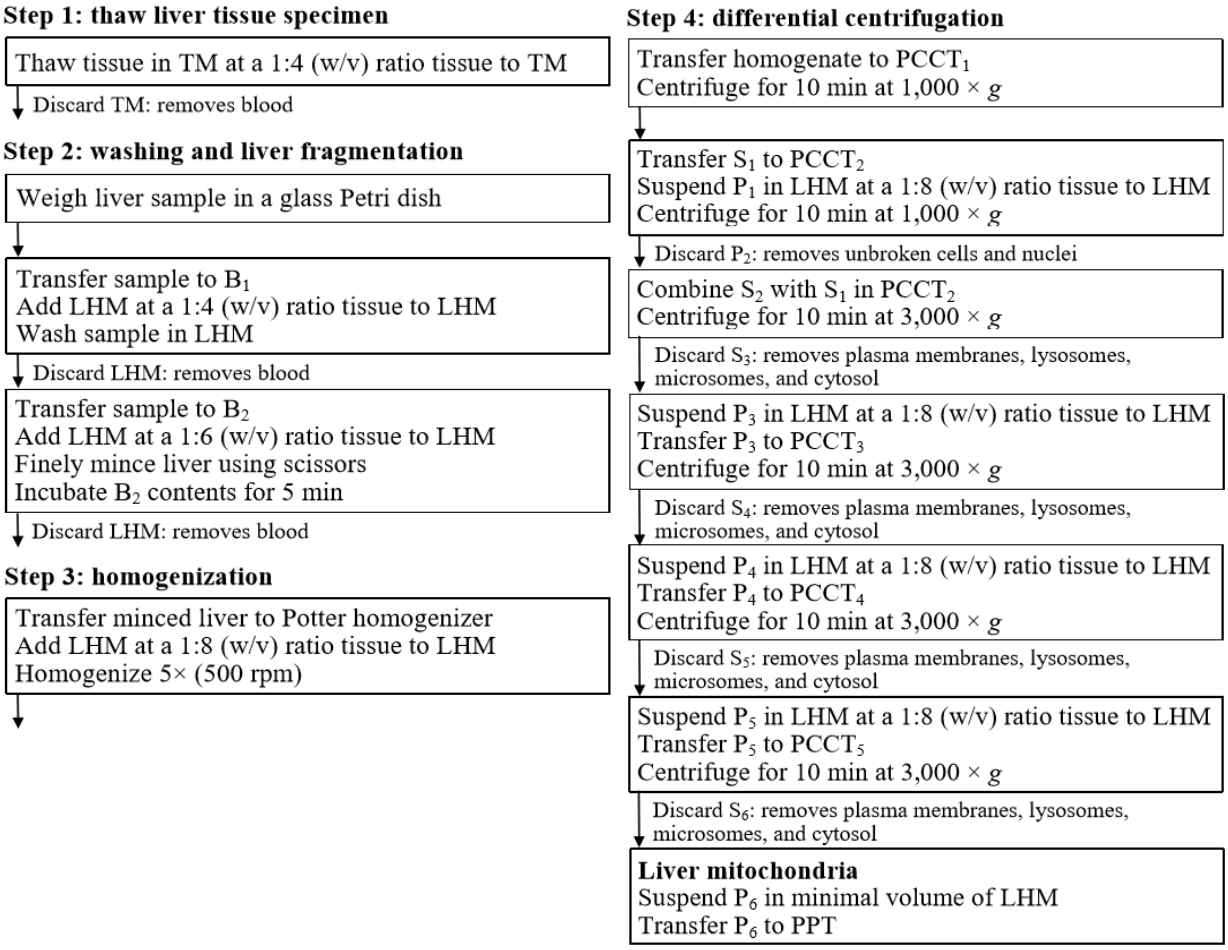

Figure 1. Schematic steps of mitochondria isolation from bovine liver adapted from Graham (1999) and Pallotti and Lenaz (2007). All solutions, glassware, centrifuge tubes, and equipment are precooled to 0 to 4°C and kept on ice throughout. TM = thawing medium; B = beaker; LHM = liver homogenization medium; PCCT = polycarbonate centrifuge tube; S = supernatant; P = pellet; and PPT = polypropylene tube.

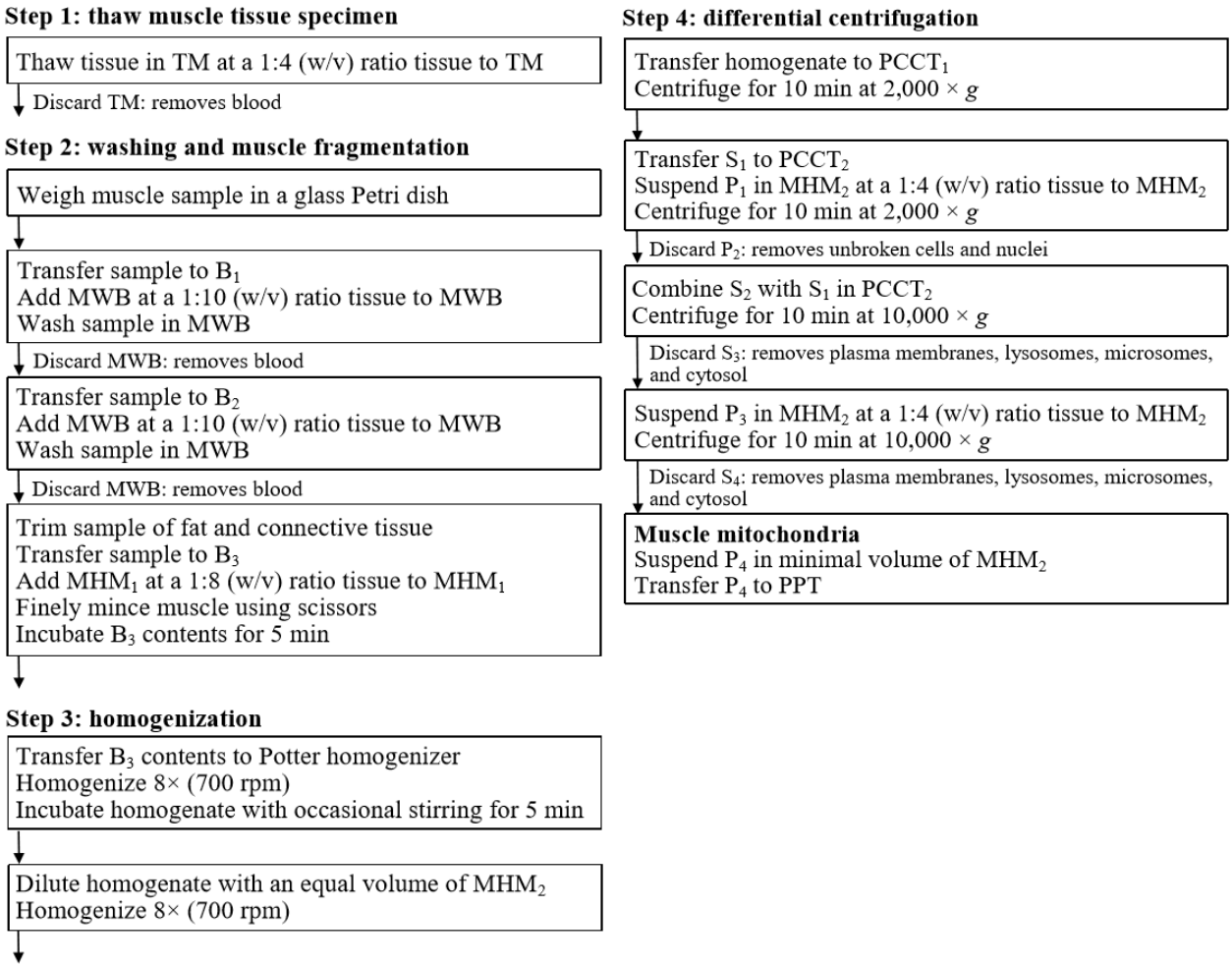

Figure 2. Schematic steps of mitochondria isolation from bovine muscle adapted from Bhattacharya et al. (1991) and Graham (1999). All solutions, glassware, centrifuge tubes, and equipment are precooled to 0 to 4°C and kept on ice throughout. TM = thawing medium; B = beaker; MWB = muscle wash buffer; MHM = muscle homogenization medium; PCCT = polycarbonate centrifuge tube; S = supernatant; P = pellet; PPT = polypropylene tube.

Table 2. Least square means for the effects of excess dietary S from supplemental sodium sulfate fed to growing-finishing steers on blood variables.

	Treatment[1]			*P*-value		
Item	LS	HS	SEM	Trt	Day	Trt × Day
Plasma, mg/L						
Cu						
Growing phase	1.07	1.06	0.04	0.83	<0.0001	0.71
Finishing phase	1.37	1.23	0.05	0.07	0.42	0.31
Zn						
Growing phase	1.73	1.75	0.06	0.74	<0.0001	0.34
Finishing phase	1.82	1.98	0.04	0.02	0.04	0.99
Total hemoglobin, g/dL						
Growing phase	11.98	12.26	0.15	0.22	<0.0001	0.22
Finishing phase	12.79	13.24	0.21	0.15	<0.0001	0.05
Sulfhemoglobin,[2] %						
Growing phase	0.29	0.34	0.01	<0.001	<0.0001	<0.001
Finishing phase	0.48	0.58	0.02	<0.001	<0.01	0.32

[1]LS = low S and HS = high S.
[2]As a percentage of total hemoglobin.

Table 3. Least square means for the effects of excess dietary S from supplemental sodium sulfate fed to growing-finishing steers on liver mineral status (dry matter basis).

	Treatment			
Item[2]	LS	HS	SEM	*P*-value
Ca, %	0.026	0.030	0.001	0.09
Mg, %	0.072	0.075	0.002	0.28
P, %	1.29	1.32	0.03	0.62
K, %	1.13	1.22	0.03	0.06
Na, %	0.23	0.23	0.02	0.86
S, %	0.72	0.72	0.02	0.85
Co, mg/kg	0.73	0.81	0.04	0.25
Cu, mg/kg	187	225	20	0.21
Fe, mg/kg	200	166	20	0.24
Mn, mg/kg	9.0	9.3	0.6	0.70
Zn, mg/kg	132	133	8	0.96

[1]LS = low S and HS = high S.
[2]Samples collected on d 234.
[3]ND = none detected.

Table 4. Least square means for the effects of excess dietary S from supplemental sodium sulfate fed to growing-finishing steers on muscle mineral status (dry matter basis).

	Treatment			
Item[2]	LS	HS	SEM	*P*-value
Ca, %	0.023	0.026	0.002	0.39
Mg, %	0.080	0.082	0.005	0.78
P, %	0.68	0.69	0.02	0.69
K, %	1.24	1.26	0.04	0.80
Na, %	0.25	0.25	0.01	0.72
S, %	0.64	0.65	0.02	0.72
Cu, mg/kg	1.7	1.6	0.1	0.33
Fe, mg/kg	56	60	3	0.50
Mn, mg/kg	ND[3]	ND		
Zn, mg/kg	144	157	12	0.48

[1]LS = low S and HS = high S.
[2]Samples collected on d 234.
[3]ND = none detected.

Table 5. Least square means for the effects of excess dietary S from supplemental sodium sulfate fed to growing-finishing steers on mitochondria protein yield and cytochrome *c* oxidase (CytOx) activity.

	Treatment[1]			
Item	LS	HS	SEM	*P*-value
Liver CytOx				
Protein yield, mg/g wet tissue	67.8	70.6	3.24	0.69
Activity, %	102.9	100.3	10.13	0.86
Muscle CytOx				
Protein yield, mg/g wet tissue	36.7	35.7	1.82	0.69
Activity, %	70.5	58.9	9.03	0.38

[1]LS = low S and HS = high S.

CHAPTER V

Effects of supplemental Cu to primiparous beef heifers consuming diets with and without S pre- and postpartum on heifer and progeny productive and physiological responses

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ABSTRACT

A study was conducted to assess the effects of supplemental Cu to primiparous beef heifers consuming Cu deficient diets with or without supplemental S pre- and postpartum on heifer and progeny productive and physiological responses. Thirty-six primiparous beef heifers (20 ± 0.5 mo of age) of predominantly Angus breeding were stratified by BW (398 ± 24.9 kg), BCS, and anticipated calving date and assigned to 12 mixed grass paddocks (3 heifers/paddock) for a 260 d study. Pens were assigned randomly to 1 of 4 treatments (2 × 2 factorial): 1) 0.15% S and 6 mg Cu/kg; 2) 0.15% S and 12 to 14 mg Cu/kg (from tribasic copper chloride); 3) 0.55% S (from sodium sulfate) and 6 mg Cu/kg; or 4) 0.55% S (from sodium sulfate) and 12 to 14 mg Cu/kg (from tribasic copper chloride) of dry matter (**DM**). Treatments were formulated to meet nutrient requirements during late gestation and lactation, with the exception of Cu and S. A cracked corn and soybean meal based supplement delivered each treatment -113 to 150 ± 16 d relative to parturition. Heifer body weight (**BW**) and body condition score were collected monthly and at parturition. Calf BW was collected at birth with subsequent BW collected monthly. Heifer mineral status was assessed in blood samples collected d -113, -85, -57, -29, 56, 85, 113 and 150 ± 16 and liver biopsy samples collected d -113, -57, 56, and 113 ± 16 relative to parturition. Progeny mineral status was assessed in blood samples collected at birth and d 31, 59, 86, 115, 141, and 150 ± 6 and liver biopsy samples collected d 7, 59, and 115 ± 6 relative to

birth. No differences ($P \geq 0.19$) were observed among treatments for measures of heifer growth performance. Progeny from heifers fed 0.15% S and 6 mg Cu/kg of DM exhibited lower birth weights (Cu × S interaction, $P = 0.09$); however, treatments did not affect ($P \geq 0.13$) other measures of progeny growth performance. Heifers fed 0.55% S exhibited lower plasma and liver Cu concentrations and plasma ceruloplasmin activity (S main effect, $P \leq 0.07$). Progeny liver Cu concentrations were similar among treatments and indicative of adequate liver Cu status in cattle, which was in contrast to maternal Cu indices. Based on heifer plasma and liver Cu status, the potential for dysfunction exists when feeding 0.55% S of DM to primiparous beef heifers consuming Cu deficient diets pre- and postpartum and the supplementation of dietary Cu greater than 12 to 14 mg Cu/kg of DM is necessary to prevent Cu status from falling to levels at or near deficiency.

Key words: beef heifer, copper, performance, physiological response, progeny, sulfur

INTRODUCTION

Nutritional management during gestation impacts progeny health and performance via fetal programming (Funston et al., 2010; Bohnert et al., 2013). Trace minerals are essential for bovine fetal development, and the fetus depends completely on the dam for an adequate supply of these elements (Hidiroglou and Knipfel, 1981; Hostetler et al., 2003). If the maternal mineral supply is inadequate, newborn offspring may have low body reserves and are susceptible to trace mineral deficiencies and decreased performance early in life (Weiss et al., 1983).

The bovine fetus has a substantial demand for Cu. There is an increase in Cu deposition in fetal tissues throughout gestation (Moss et al., 1974; Graham et al., 1994), and Cu is required for the proper development of the fetal nervous, reproductive, and immune systems (Hostetler et al., 2003). The Cu antagonists S, Mo, and Fe induce Cu deficiency by forming insoluble Cu complexes in the digestive tract, blood stream, and tissues of ruminants (Dick et al., 1975; Suttle

and Field, 1983; Allen and Gawthorne, 1987). Results of feeding diets deficient in Cu with supplemental dietary antagonists to Cu on the productive and physiological responses of beef heifers and their offspring are limited. Gengelbach et al. (1994) reported no difference in heifer calving weight or postpartum weight change, whereas calves experienced retardation in growth when inducing Cu deficiency in heifers by supplementing the heifer's marginally Cu deficient diet with Mo. Muehlenbein et al. (2001) reported no difference in the postpartum weight change of heifers, calf weaning weights, or in 60-d postpartum pregnancy rates when inducing Cu deficiency in heifers and progeny by supplementing the heifer's marginally Cu deficient diet with Fe and Mo.

No studies have been conducted to assess the effects of supplemental Cu to primiparous beef heifers consuming Cu deficient diets with or without supplemental S pre- and postpartum on heifer and progeny productive and physiological responses. It was hypothesized that feeding heifers marginally deficient Cu diets with supplemental S pre- and postpartum would be detrimental to heifer and progeny physiological responses and heifer reproductive performance. Moreover, it was hypothesized that supplemental Cu to primiparous beef heifers consuming Cu deficient diets with or without supplemental S pre- and postpartum would improve heifer and progeny physiological responses and heifer reproductive performance. Therefore, the purpose of this study was to assess the effects of supplemental Cu to primiparous beef heifers consuming Cu deficient diets with or without supplemental S pre- and postpartum on heifer and progeny productive and physiological responses.

MATERIALS AND METHODS

Animal handling procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (protocol #14015) and followed guidelines recommended in

the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010). The study was conducted at the University of Arkansas Stocker Cattle Receiving and Backgrounding Unit (Savoy, AR).

Animals, experimental design, and treatments

Thirty-six primiparous beef heifers (20 ± 0.5 mo of age) of primarily Angus breeding were transported from the University of Arkansas Cow-Calf Unit (Savoy, AR) at approximately 170 d of gestation. Upon arrival, heifers were vaccinated against respiratory and gastrointestinal diseases (Vira Shield 6 + VL5; Elanco Animal Health, Greenfield, IN) and treated for external parasites (StandGuard; Elanco Animal Health). Heifers were stratified by body weight (**BW**; 398 ± 24.9 kg), body condition score (**BCS**), and anticipated calving date and assigned to 12 paddocks (3 heifers/paddocks) for a 260-d maternal nutrition study. Paddocks were assigned randomly to 1 of 4 treatments (2 × 2 factorial): 1) 0.15% S and 6 mg Cu/kg; 2) 0.15% S and 12 to 14 Cu/kg (from tribasic copper chloride; IntelliBond C; Micronutrients, Indianapolis, IN); 3) 0.55% S (from sodium sulfate; Prince Agri Products, Inc., Quincy, IL) and 6 mg Cu/kg; or 4) 0.55% S (from sodium sulfate) and 12 to 14 mg Cu/kg (from tribasic copper chloride; Table 1) of dry matter (**DM**). Diets were formulated using individual ingredient composition analysis before the start of the study (method 975.03; AOAC, 1988; Altheimer Laboratory, Fayetteville, AR). Moreover, diets were formulated to meet or exceed primiparous heifer nutrient requirements during late gestation, and subsequent early lactation, with the exception of Cu and S (NRC, 2000). A cracked corn and soybean meal based supplement delivered each treatment -113 to 150 ± 16 d relative to parturition. Because the study encompassed lactation, feeding levels were increased to meet the nutrient demands of lactation. Heifers grazed mixed grass (0.18% S, 5.1 mg Cu/kg, 2.8 mg Mo/kg, 178 mg Fe/kg, 50 mg Zn/kg, 12.0% crude protein, 34.6% acid

detergent fiber, 70.4% NDF, and 8.0% ash, DM basis) paddocks (2.4-ha/paddocks). Throughout the study, heifers had free access to water and were fed once a day at 0800 h in their respective paddocks (0.5 m or more bunk space/heifer), with refusals collected and weighed before feeding. Due to bunk design and ration delivery, it was impossible to restrict treatment access by calves, thus calves may have consumed maternal supplements.

Supplement samples were collected daily and composited over 28 d periods. Forage samples (simulated grazed) were obtained every 28 d. Forage intake was not quantified. Supplement and forage samples were dried in a forced-air oven (72 h at 55°C), ground using a Wiley mill (1-m screen; Arthur H. Thomas, Philadelphia, PA), and analyzed in duplicate for DM (method 934.01; AOAC, 2000), ash (method 924.05; AOAC, 1990), crude protein (method 990.03; AOAC, 1995; Elementar Americas, Inc., Mt. Laurel, NJ), neutral and acid detergent fiber (Ankom Technology methods 5 and 6, respectively; ANKOM^{200} Fiber Analyzer; ANKOM Technology Corp., Fairport, NY), and complete minerals (method 975.03; AOAC, 1988; Altheimer Laboratory, Fayetteville, AR). Representative samples of the refusals of each pen were dried in a forced-air oven (72 h at 55°C) to calculate DM and correct nutrient intake. Intakes were calculated based on DM offered after subtracting DM refused.

Heifer performance

Initial heifer BW was determined by taking the average preprandial BW measured on 2 consecutive days (d -113 and -112 ± 16 relative to parturition) at treatment initiation. In addition, palpable BCS (1 = emaciated to 9 = obese; Wagner et al., 1988) was assessed at the start of dietary treatments. Subsequent BW and BCS were measured monthly, with BCS assessment conducted by the same trained study personnel at all time points throughout the study. Final BW was determined by taking the average preprandial BW measured on 2 consecutive days (d 149

and 150 ± 16 relative to parturition) at the end of the study. In addition, parturition BW and BCS were assessed within 24 h of parturition without feed or water restriction. Heifers failing to calve (0.15% S and 6 mg Cu/kg, *n* = 2 and 0.55% S and 6 mg Cu/kg, *n* = 1) were removed from the data set. Heifers that died, gave birth to a stillborn calf, or experienced neonatal mortality (0.15% S and 6 mg Cu/kg, *n* = 1; 0.15% S and 12 to 14 mg Cu/kg, *n* = 2; and 0.55% S and 6 mg Cu/kg, *n* = 1) were removed from the study, but their data remained in the data set.

Preweaning progeny performance

Once labor began and either the allantois or amnion presented, heifers were given 1 h to calve or make significant progress as indicated by presentation of the fetus before assistance was given. Within 24 h of parturition, calves were observed to ensure they nursed, weighed, uniquely identified, castrated if male using elastrator bands (Agri-Pro Enterprises-Iowa Inc., Iowa Falls, IA), and assigned calving ease (1 = unassisted birth to 5 = abnormal presentation), calf vigor (1 = alert and active to 5 = listless and unresponsive), and agility (1 = moves well and correct posture to 5 = significant stiffness in gate and arch in topline) scores (Vandervelde et al., 1990). Calves were returned to treatment paddocks. Subsequent BW was measured monthly throughout the study. Final progeny BW was determined by taking the average preprandial BW measured on 2 consecutive days (d 149 and 150 ± 6 relative to birth) at the end of the study. One heifer (0.55% S, no added Cu treatment) gave birth to a stillborn calf. Calf birth BW and calving ease score were recorded and retained in the data set.

Blood and liver tissue collection and analysis

Jugular blood was collected in K_2-EDTA tubes (BD Vacutainer; Becton Dickinson Co.) from all heifers d -113 and 150 ± 16 relative to parturition and 3 randomly selected calves from each treatment d 150 ± 6 relative to birth for serum Se analysis. Tubes were inverted several times to mix contents and shipped on ice to a commercial laboratory (California Animal Health

& Food Safety Laboratory, Davis, CA) for the analysis of serum Se concentration. Jugular blood was collected in sodium heparin tubes (BD Vacutainer; Becton Dickinson Co., Franklin Lakes, NJ) from all heifers at parturition and d -113, -85, -57, -29, 56, 85, 113 and 150 ± 16 relative to parturition and calves at birth and d 31, 59, 86, 115, 141, and 150 ± 6 relative to birth for plasma mineral and ceruloplasmin activity analysis. Tubes were inverted several times to mix contents and placed on ice before centrifugation (1,200 × *g*, 20 min at 20°C). Plasma was aliquoted and stored at -80°C for later analysis. Plasma was prepared for mineral analysis with a 1:9 (vol/vol) dilution of plasma in 1 *N* trace mineral-grade nitric acid. Samples were vortexed vigorously to precipitate protein and centrifuged (1,200 × *g*, 20 min at 20°C). Supernatant was collected and analyzed for complete minerals by inductively coupled plasma atomic emission spectroscopy at a commercial laboratory (method 975.03; AOAC, 1988; Altheimer Laboratory, Fayetteville, AR). Plasma ceruloplasmin activity was determined as described by Houchin (1958). Briefly, 0.1 mL of plasma (heated to 37°C) was added to1 mL of fresh 0.1% paraphenylenediamine (Sigma-Aldrich, St. Louis, MO) and incubated (30 min at 37°C). The reaction was stopped by the addition of 5 mL of cold 0.02% sodium azide (Sigma-Aldrich). Samples were vortexed and analyzed spectrophotometrically (UV-1201; Shimadzu, Kyoto, Japan) at 525 nm. Ceruloplasmin activity was expressed as milligrams per deciliter via the equation $y = -1.7 + 150x$, where y = plasma ceruloplasmin concentration (mg/dL) and x = ceruloplasmin activity in optical density units (Scheinberg et al., 1957).

Liver tissue was collected from all heifers on d -113, -57, 56, and 113 ± 16 relative to parturition and calves on d 7, 59, and 115 ± 6 relative to birth for mineral analysis. The biopsy site was aseptically prepared and locally anesthetized with lidocaine (20 mg/mL; Lido-epi; Radix Labs, Eau Claire, WI). Percutaneous biopsies were obtained through a puncture incision made

between the 11 and 12th ribs on the perceptual line from the tuber coxae to the elbow using a biopsy needle (Tru-Cut; LVWR Scientific Products Corp., Seattle, WA). Tissue samples were placed in 12 × 75-mm disposable borosilicate glass tubes (Fisher Scientific, Waltham, MA) and capped. Liver samples were dried in a forced-air oven (48 h at 50°C), digested in 1 mL trace mineral-grade nitric acid, and brought to a final volume of 5 mL with deionized water. Complete liver mineral analysis was conducted as described for plasma mineral analysis.

Estrus synchronization and breeding

At d 69 ± 16 relative to parturition, reproductive tract scores (**RTS**; 1 = immature to 5 = luteal phase; Anderson et al., 1991) were assigned to all heifers via transrectal ultrasonography (Ibex Pro; L6.2 transducer; E.I. Medical Imaging, Loveland, CO). Seven days later, all heifers were synchronized for ovulation with the 7-d controlled intravaginal drug release (**CIDR**) insert and prostaglandin protocol for beef cattle. The protocol consisted of heifers receiving a CIDR (Eazi-Breed CIDR; Zoetis, Parsippany, NJ) device containing 1.38 g of progesterone at protocol initiation and CIDR removal 7 d later concurrent with an intramuscular injection of 25 mg of prostaglandin F2α (5 mL Lutalyse; dinoprost tromethamine; Zoetis). Heifers were visually detected for estrus twice daily with the aid of a heat detection patch (Estrotect Heat Detectors; Rockway Inc., Spring Valley, WI) affixed to the tailhead. Estrus was defined to have occurred when a heat detection patch was greater than 50% colored (i.e., activated). Heifers detected in estrus within 72 h after prostaglandin F2α administration were artificially inseminated based on the morning/evening rule. Heifers not observed in standing heat were retreated 10 to 13 d postestrus using the same 7-d CIDR insert and prostaglandin protocol to resynchronize repeat estrus and artificial insemination. Pregnancy confirmation was carried out 30 d postbreeding via transrectal ultrasonography.

Statistical analysis

Heifer and progeny growth performance and mineral status data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Inst., Inc., Cary, NC) by means of a variance components covariance structure. Denominator degrees of freedom were estimated using the Kenward-Rogers option. Data adherence to the assumptions of the statistical test was established. Repeated measures analyses were used to analyze the effects of dietary treatment on heifer and progeny mineral status. Fixed effects included in the model were dietary treatment and day, as well as the 2-way interaction, whereas paddock (experimental unit) was included as a random effect. Day was included as a repeated effect. The effects of dietary treatment on heifer BCS and reproductive performance and progeny calving ease, calf vigor, and agility scores were analyzed using the GLIMMIX procedure of SAS by means of a multinomial distribution, cumulative logit link function, and variance components covariance structure. Paddock (experimental unit) was included in the model as a random effect. Orthogonal contrasts were used to assess the effects of Cu vs. S supplementation. Statistical significance was declared at $P \leq 0.10$. Means reported in tables are least squares means.

RESULTS AND DISCUSSION

The dietary treatments were formulated to investigate the effects of supplemental Cu to primiparous beef heifers consuming Cu deficient diets with or without supplemental S pre- and postpartum on heifer and progeny productive and physiological responses. Analyzed S, Cu, Mo, and Fe concentrations of individual supplement ingredients are in Table 1. Diets without supplemental Cu averaged 6.1 mg Cu/kg of DM, whereas those with supplemental Cu averaged 10.9 mg Cu/kg of DM, slightly below the target of 12 to 14 mg Cu/kg of DM. Diets without supplemental S averaged 0.20% S of DM, whereas those with supplemental S averaged 0.58% S

of DM, both slightly above the target values of 0.15 and 0.55% S of DM, respectively. Although the Cu and S analyses indicated that the dietary treatments varied somewhat from targeted values, the treatments were delivered to animals close to the design formulations.

Heifer and progeny growth performance

No differences ($P \geq 0.19$) were observed among treatments for heifer BW, BW change, ADG, or BCS throughout the study (Table 2). Progeny from heifers fed 0.15% S and 6 mg Cu/kg of DM exhibited lower birth weights (Cu × S interaction, $P = 0.09$); however, treatments did not affect ($P \geq 0.13$) progeny final BW, ADG, and calving ease, calf vigor, and agility scores (Table 3). Consistent with these results, Gengelbach et al. (1994) reported that feeding primiparous beef heifers Cu deficient diets with supplemental dietary Cu antagonists pre- and postpartum did not affect heifer growth performance. In their study, supplementing 5 mg Mo/kg or 600 mg Fe/kg of DM to a diet containing 4.0 mg Cu/kg, 0.32% S, 1.3 mg Mo/kg, and 192 mg Fe/kg of DM did not affect postpartum weight change. However, progeny from heifers fed elevated levels of Mo experienced decreased weight gain. The reduced progeny weight gain observed in their study is consistent with other studies with growing calves in which the addition of 5 mg Mo/kg of DM decreased weight gain, whereas supplemental Fe had no effect on gain when the Cu status was similar between treatments (Humphries et al., 1983; Phillippo et al., 1987). These results suggest that the reduced weight gain of progeny from heifers fed elevated levels of Mo was likely due to excess dietary Mo rather than Cu deprivation. In the current study, the reason progeny from heifers fed 0.15% S and 6 mg Cu/kg of DM exhibited lower birth weights remains unknown as it occurred in the absence of sufficient dietary S to reduce Cu absorption and in the absence of differences in heifer growth performance between treatments.

Copper supplementation at 12 to 14 mg Cu/kg of DM to heifers fed 0.15 or 0.55% S of DM pre- and postpartum did not affect heifer and progeny growth performance. Consistent with

these results, others studies have observed that supplementing additional dietary Cu as a strategy to overcome the effects of Cu deficient diets with Cu antagonists does not affect cattle growth performance responses. Muehlenbein et al. (2001) reported supplementing 100 or 200 mg Cu/kg of DM to a hay-based diet containing 5.1 mg Cu/kg, 5.1 mg Mo/kg, and 411 mg Fe/kg of DM pre- and postpartum did not affect primiparous beef heifer and progeny growth performance. Similarly, Bailey et al. (2001) observed that growing beef heifer performance was not affected when supplementing 22 or 49 mg Cu/kg of DM to a diet containing 6.05 mg Cu/kg, 0.29% S, 12.2 mg Mo/kg, and 451 mg Fe/kg of DM during a 112-d study.

Heifer and progeny mineral status

Sulfur status. Heifer plasma S concentrations decreased ($P < 0.10$; Table 4) throughout the study regardless of treatment; however, heifers fed 0.55% S of DM exhibited greater (S main effect, $P = 0.03$) plasma S concentrations during the postpartum period compared to heifers fed 0.15% S of DM. The greater plasma S concentrations during the postpartum period for heifers fed 0.55% S of DM may be attributed to the combined effects of dietary S supplementation and S no longer being deposited into a growing fetus (Hansard and Mohammed, 1969). Changes in heifer plasma S concentrations did not coincide with liver S concentrations, as no difference ($P = 0.84$; Table 5) occurred in liver S concentrations among treatments at any sampling time. Heifer supplementation affected progeny plasma S concentrations. Progeny from heifers fed 12 to 14 mg Cu/kg of DM exhibited lower (Cu main effect, $P = 0.04$; Table 6) plasma S concentrations at birth than progeny from heifers fed 6 mg Cu/kg of DM. The lower plasma S concentrations in progeny from heifers fed 12 to 14 mg Cu/kg of DM may relate to a depletion of S due an interaction with Cu (Suttle, 1974). Progeny plasma S concentrations declined ($P < 0.10$) after birth regardless of treatment, and progeny had similar ($P = 0.67$) preweaning plasma S

concentrations among treatments. Heifer supplementation did not affect ($P = 0.90$; Table 7) progeny liver S concentrations. No differences ($P = 0.95$) were observed among treatments for progeny liver S concentrations at birth. Consistent with progeny plasma S concentrations, liver S concentrations declined ($P < 0.10$) after birth regardless of treatment, and no differences ($P = 0.79$) were detected among treatments for preweaning liver S concentrations.

Copper status. No differences ($P = 0.56$) were detected among treatments for initial heifer plasma Cu concentrations; however, all treatments exhibited plasma Cu concentrations consistent with marginal deficiency (0.5 to 0.7 mg Cu/mL; Kincaid, 2000) in cattle. The possible reason for the marginal plasma Cu concentrations was the demand of the fetus. The developing fetus has a great demand for Cu, particularly during the last trimester, as Cu is essential for fetal growth and development (Gooneratne and Christensen, 1989). Heifers fed 0.55% S of DM exhibited decreased (S main effect, $P \leq 0.07$) plasma Cu concentrations indicative of deficiency (0.2 to 0.5 mg Cu/mL; Kincaid, 2000) and plasma ceruloplasmin activity during the prepartum, parturition, and postpartum periods compared to heifers fed 0.15% S of DM. Increased plasma Cu concentrations are often observed in pregnant cattle due to the greater rate of ceruloplasmin synthesis resulting from increased estrogen levels during late pregnancy (Yokus and Cakir, 2006). The lower plasma Cu concentrations and ceruloplasmin activity in heifers fed 0.55% S of DM can be attributed to reduced Cu bioavailability due to the formation of insoluble CuS in the gut (Suttle, 1974). Similar to heifers fed 0.55% S of DM, heifers fed 0.15% S of DM had plasma Cu concentrations considered marginal (0.5 to 0.7 mg Cu/mL; Kincaid, 2000) for cattle throughout the study. The low plasma Cu status for all treatments may explain why heifer growth performance data were not affected by treatment.

No differences ($P = 0.96$) were detected among treatments for initial heifer liver Cu concentrations, indicating that all treatments had similar and adequate (125 to 600 mg Cu/kg of DM; Kincaid, 2000) liver Cu status at the beginning of the study. Heifers fed 0.55% S of DM experienced a decline ($P < 0.10$) in liver Cu concentrations over the course of the study. In addition, heifers fed 0.55% S of DM had lower (S main effect, $P = 0.06$) liver Cu concentrations postpartum compared to heifers fed 0.15% S of DM. However, heifers fed 12 to 14 mg Cu/kg of DM experienced increased liver Cu concentrations (Cu main effect, $P = 0.08$) postpartum compared to heifers fed 6 mg Cu/kg of DM. Heifers fed 0.55% S of DM had marginal (30 to 125 mg Cu/kg of DM; Wikse et al., 1992) liver Cu concentrations during the pre- and postpartum periods, whereas heifers fed 0.15% S and 12 to 14 mg Cu/kg of DM experienced adequate liver Cu concentrations throughout the study. The inability of heifers fed 0.55% S of DM to regain adequate plasma and liver Cu status after parturition indicates that their body Cu reserves were depleted prepartum, and that the ability of S to inhibit Cu absorption and interfere with Cu metabolism would not allow the animals to become Cu-replete. These results suggest that when based on plasma and liver Cu status, the potential for dysfunction exists when feeding 0.55% S of DM to primiparous beef heifers consuming Cu deficient diets pre- and postpartum and dietary Cu supplementation greater than 12 to 14 mg Cu/kg of DM is necessary to prevent Cu status from falling to levels at or near deficiency.

Heifer supplementation did not affect progeny plasma Cu concentrations, plasma ceruloplasmin activity, or liver Cu concentrations. Progeny plasma Cu concentrations were similar ($P = 0.94$) among treatments at birth, but plasma Cu concentrations were indicative of clinical deficiency (< 0.2 mg Cu/kg of DM; Kincaid, 2000) for cattle. Progeny plasma Cu concentrations increased ($P < 0.10$) after birth to values indicative of deficiency in cattle, but no

differences ($P = 0.36$) were noted among treatments for preweaning plasma Cu concentrations. Consistent with plasma Cu concentrations, no differences ($P = 0.53$) were detected among treatments for progeny plasma ceruloplasmin activity at birth. Moreover, plasma ceruloplasmin activity increased ($P < 0.10$) after birth regardless of treatment, and preweaning plasma ceruloplasmin activity was similar ($P = 0.69$) among treatments. No differences ($P \geq 0.19$) were observed among treatments for progeny liver Cu concentrations at birth and preweaning. In contrast to progeny plasma Cu concentrations, liver Cu concentrations at birth and preweaning were indicative of adequate liver Cu status in cattle. The bovine neonate depends heavily on liver Cu stores for postnatal utilization because of the delayed synthesis of ceruloplasmin (Chang et al., 1975) and the fact that cow milk is not a significant source of Cu (Underwood and Suttle, 1999). Prior research has indicated that fetal liver Cu concentrations at birth should be at least 300 mg Cu/kg of DM to prevent Cu deficiency during the early months of life (Gooneratne and Christensen, 1989). Because all heifers, regardless of treatment, were assessed as at or near Cu deficiency during the prepartum period by their plasma Cu levels, it is possible that they did not mobilize enough Cu to provide the fetus with normal liver Cu reserves (Xin et al., 1993). As a result, their progeny were much more susceptible to Cu deficiency which may explain the deficient progeny plasma Cu status. Similarly, Gooneratne et al. (1986) reported pregnant cows fed a 0.35% S and 5 mg Cu/kg of DM diet became severely Cu deficient and gave birth to calves with low plasma Cu concentrations, plasma ceruloplasmin activity, and liver Cu stores. However, prior research has also suggested that the fetus has the ability to accrue Cu from its dam even though the dam is potentially Cu deficient (Gooneratne et al., 1989). This may explain why progeny liver Cu concentrations were similar among treatments and indicative of adequate liver Cu status, which was in contrast to maternal Cu indices.

Zinc status. All heifers, regardless of treatment, exhibited adequate (0.8 to 1.4 mg Zn/mL; Kincaid, 2000) plasma Zn concentrations at the beginning of the study; however, plasma Zn concentrations declined ($P < 0.10$), regardless of treatment, to values indicative of marginal deficiency (0.5 to 0.8 mg Zn/mL; Kincaid, 2000) by parturition. These results may be attributed to the increase in maternal glucocorticoids associated with parturition (Smith et al., 1973). Glucocorticoids reduce Zn absorption and stimulate metallothionein synthesis, which pulls Zn into cells (Kincaid, 2008). Heifers fed 0.15% S and 12 to 14 mg Cu/kg of DM exhibited decreased (Cu × S interaction; $P = 0.07$) plasma Zn concentrations during the parturition period. The reason for this result is unknown. Heifers fed 12 to 14 mg Cu/kg of DM experienced decreased parturition liver Zn concentrations (Cu main effect, $P = 0.03$) compared to heifers fed 6 mg Cu/kg of DM. Decreased parturition liver Zn concentrations for heifers fed additional dietary Cu may be attributed to the combined effects of Cu inhibition of Zn intestinal absorption (Abdel-Mageed and Oehme, 1991) and the need for Zn in colostrum synthesis (Kincaid, 2008). All heifers, regardless of treatment, had adequate (25 to 200 mg Zn/kg of DM; Kincaid, 2000) liver Zn concentrations throughout the study. Heifer supplementation did not affect ($P = 0.33$) progeny plasma Zn concentrations. Progeny plasma Zn concentrations were similar ($P = 0.58$) among treatments at birth, but plasma Zn concentrations were generally indicative of deficiency (0.2 to 0.4 mg Zn/mL; Kincaid, 2000) for cattle. Progeny plasma Zn concentrations numerically increased after birth to values indicative of marginal deficiency in cattle, and preweaning plasma Zn concentrations were similar ($P = 0.49$) among treatments. Heifer supplementation affected progeny liver Zn concentrations. Progeny from heifers fed 0.15% S and 6 mg Cu/kg of DM had decreased (Cu × S interaction; $P = 0.06$) plasma Zn concentrations at birth. The reason for this result is unknown as it occurred in contrast to maternal Zn indices. Progeny preweaning liver Zn

concentrations were similar ($P = 0.80$) among treatments. All progeny, regardless of treatment, experienced adequate liver Zn concentrations throughout the study.

Selenium status. All heifers, regardless of treatment, exhibited adequate (0.08 to 0.3 mg Se/L; Kincaid, 2008) serum Se concentrations throughout the study. Heifers fed 0.15% S of DM experienced increased ($P < 0.10$) serum Se concentrations postpartum, but no differences ($P = 0.54$) were observed among treatments for postpartum serum Se concentrations. Selenium and S have similar physical and chemical properties, and a number of studies indicate that increasing dietary S reduces the bioavailability of Se (Spears, 2003). Accordingly, the increased serum Se concentrations for heifers fed 0.15% S of DM suggests that insufficient dietary S was available to reduce Se bioavailability. Heifer supplementation affected progeny serum Se concentrations. Progeny from heifers fed 0.55% S of DM experienced decreased (S main effect, $P = 0.04$) serum Se concentrations compared to progeny from heifers fed 0.15% S of DM; however, all progeny, regardless of treatment, exhibited adequate serum Se concentrations. The decreased serum Se concentrations for progeny from heifers fed 0.55% S of DM suggests the depletion of Se due an interaction with S.

Molybdenum status. Heifers fed 12 to 14 mg Cu/kg of DM had decreased prepartum liver Mo concentrations (Cu main effect, $P = 0.07$) compared to heifers fed 6 mg Cu/kg of DM. Dietary Mo can inhibit the uptake and utilization of Cu. In the rumen, Mo combines with reduced S to form thiomolybdate compounds that bind Cu and prevent Mo absorption (Gould and Kendall, 2011). Accordingly, heifers fed 12 to 14 mg Cu/kg of DM may have limited liver Mo accumulation due to the formation of thiomolybdate compounds that readily bound Cu. Consistent with these results, prior research has indicated that trace mineral supplements containing a combination of inorganic and amino acid-complexed forms of Cu limited Mo

accumulation in the liver (Bailey et al., 2001). No differences ($P = 0.58$) occurred in postpartum liver Mo concentrations among treatments. Heifer supplementation did not affect ($P = 0.79$) progeny liver Mo concentrations. Because no nutritional deficiencies of Mo under practical conditions have been reported in ruminants, no estimates are available on the minimum values for Mo in blood and tissues (Kincaid, 2000).

Heifer reproductive performance

Synchronized estrus response was not affected ($P \geq 0.81$) by treatments; however, RTS and synchronized conception rates were greater (Cu main effect, $P \leq 0.07$) for heifers fed 12 to 14 mg Cu/kg of DM compared to heifers fed 6 mg Cu/kg of DM (Table 8). Repeated trace mineral injections have been found to not affect RTS (Stokes et al., 2018). Accordingly, the reason heifers fed 12 to 14 mg Cu/kg of DM exhibited greater RTS is unknown. Imbalances between the production of reactive oxygen species (**ROS**) and the action of antioxidant systems results in DNA damage and cell apoptosis. Low *in vitro* ROS concentrations promote oocyte developmental competence and impacts subsequent embryo development (Blondin et al., 1997; Combelles et al., 2009), whereas high ROS concentrations alter normal cellular function by inducing the oxidative damage of intracellular components and apoptosis (Guerin et al., 2001). Therefore, the antioxidant role of Cu could be an important mechanism in preventing oxidative damage in the cumulus cells of bovine oocytes.

Optimal embryo development to the blastocyst stage has been demonstrated to be partially dependent on the presence of adequate Cu concentrations during *in vitro* maturation. Picco et al. (2012) reported that supplemental Cu during the *in vitro* maturation of bovine oocytes increased the DNA integrity of cumulus cells. Similarly, Rosa et al. (2016) observed that Cu supplementation to *in vitro* maturation medium decreased DNA damage and apoptosis in

bovine cumulus cells and improved the subsequent development of preimplantation embryos. Therefore, the improved reproductive performance of heifers fed 12 to 14 mg Cu/kg of DM in the current study may be due to a greater capacity for oocyte development and the subsequent development of preimplantation embryos due to the presence of adequate Cu concentrations.

Prior research has established that the common practice of overfeeding dietary protein to high producing dairy cows to sustain milk production increases concentrations of plasma urea nitrogen and decreases uterine pH on d 7 of the estrous cycle when compared with those fed a balanced diet (Elrod et al., 1993; Elrod and Butler, 1993). Similar to high dietary protein intake, high dietary S intake has been reported to decrease uterine pH during the luteal phase. Perry et al. (2009) reported a negative correlation between concentrations of blood sulfate and uterine pH in heifers on d 7 and 11 of the estrous cycle. As plasma sulfate concentrations increased, uterine pH decreased. Changes in uterine pH during the midluteal phase are important due to its effects on embryo development and survival. Ocon and Hansen (2003) observed that in vitro culture of bovine embryos at a pH less than 7.0 decreased cleavage rates and development to the blastocyst stage. Accordingly, it was hypothesized that the heifers fed 0.55% S of DM would experience changes in uterine pH that decreased reproductive performance. However, these treatments increased heifer postpartum plasma S concentrations without a concomitant decrease in reproductive performance. These results are consistent with Grant et al. (2013) who reported that high S intake (from $CaSO_4$) increased concentrations of plasma sulfate, but that the increased concentrations of plasma sulfate were not associated with a decrease in uterine pH. The authors attributed these results to the alkalinizing capacity of the additional dietary Ca from the $CaSO_4$ used in the experiment to mitigate the effect of decreased uterine pH. Similarly, the increased heifer postpartum plasma S concentrations without a concomitant decrease in reproductive

performance observed in the current study may be attributed to the use of Na_2SO_4 (nonacidic dietary S source) to increase the concentration of dietary S.

The purpose of this study was to assess the effects of supplemental Cu to primiparous beef heifers consuming Cu deficient diets with or without supplemental S pre- and postpartum on heifer and progeny productive and physiological responses. The results of this study indicate that supplementation of dietary Cu up to 12 to 14 mg Cu/kg of DM to overcome the effects of feeding 0.55% S of DM to primiparous beef heifers pre- and postpartum did not affect heifer or progeny growth performance responses. Furthermore, based on plasma and liver Cu status, the potential for dysfunction exists when feeding 0.55% S of DM to primiparous beef heifers consuming Cu deficient diets pre- and postpartum, and the supplementation of dietary Cu greater than 12 to 14 mg Cu/kg of DM is necessary to prevent Cu status from falling to levels at or near deficiency. The results of this study also support earlier work that has demonstrated that the fetus has the ability to accrue Cu from its dam even though the dam is potentially Cu deficient. Progeny liver Cu concentrations were similar among treatments and indicative of adequate liver Cu status in cattle, which was in contrast to maternal Cu indices.

ACKNOWLEDGMENTS

The authors thank J. Reynolds, J. Hornsby (retired), D. Bignar, D. Galloway, and T. Johnson (University of Arkansas, Department of Animal Science, Fayetteville, AR) for assistance with animal care and data and specimen collection. Authors are also appreciative to R. Rorie (University of Arkansas, Department of Animal Science, Fayetteville, AR) for assistance with estrus synchronization and artificial insemination.

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Table 1. Ingredient and analyzed composition of treatments fed to primiparous beef heifers from -113 to 150 ± 16 d relative to parturition.

	Treatment[1,2,3]			
	0.15% S		0.55% S	
Item	No added Cu	Added Cu	No added Cu	Added Cu
Ingredient, as-fed basis				
Cracked corn, %	86.5	86.5	75.1	75.1
Soybean meal, %	8.0	8.0	10.0	10.0
Salt, %	1.0	1.0	1.0	1.0
Limestone, %	1.9	1.9	1.9	1.9
Vitamin A, D, E premix,[4] %	0.1	0.1	0.1	0.1
Rumensin premix,[5] %	0.4	0.4	0.4	0.4
Sodium sulfate (anhydrous), %	--	--	9.4	9.4
Trace mineral premix A[6]	+	--	+	--
Trace mineral premix B[7]	--	+	--	+
Molasses, %	2.5	2.5	2.5	2.5
Analyzed composition[8]				
CP, %	11.5	12.0	12.9	12.5
ADF, %	2.5	2.2	2.1	2.1
NDF, %	10.7	10.7	9.2	8.5
S, %	0.30	0.21	2.21	2.29
Cu, mg/kg	6	29	14	40
Mo, mg/kg	ND[9]	ND	ND	ND
Fe, mg/kg	131	130	172	169

Table 1 (Cont.)

	Treatment[1,2,3]			
	0.15% S		0.55% S	
Item	No added Cu	Added Cu	No added Cu	Added Cu
Zn, mg/kg	100	114	140	136

[1]Fed at a rate of 1.8 kg·d^{-1}·hd^{-1} to heifers grazing mixed grass pasture. Feeding levels were increased to a rate of 2.1 kg·d^{-1}·hd^{-1} to meet the nutrient demands of lactation.
[2]No added Cu provided 6 mg Cu/kg of DM. Added Cu provided 12 to 14 mg Cu/kg of DM.
[3]Total dietary provision: 0.15% S, no added Cu = 0.21% S, 5.3 mg Cu/kg, 2.2 mg Mo/kg, 168 mg Fe/kg, and 60 mg Zn/kg and 0.15% S, added Cu = 0.19% S, 10.1 mg Cu/kg, 2.2 mg Mo/kg, 168 mg Fe/kg, and 63 mg Zn/kg. When corrected for orts [amount refused (orts) subtracted from amount offered], total dietary provision: 0.55% S, no added Cu = 0.57% S, 6.8 mg Cu/kg, 2.2 mg Mo/kg, 177 mg Fe/kg, and 66 mg Zn/kg and 0.55% S, added Cu = 0.58% S, 11.7 mg Cu/kg, 2.2 mg Mo/kg, 177 mg Fe/kg, and 65 mg Zn/kg.
[4]Provided when fed at 1.8 kg/d: 8,890 IU vitamin A; 1,780 IU vitamin D; and 1.1 IU vitamin E.
[5]Rumensin (Elanco Animal Health; Indianapolis, IN) provided 88 mg of monensin when fed at 1.8 kg/d.
[6]Provided when fed at 1.8 kg/d: 0.5 mg Co; 2.5 mg I; 100 mg Mn; 0.5 mg Se; and 150 mg Zn.
[7]Provided when fed at 1.8 kg/d: 0.5 mg Co; 35 mg Cu; 2.5 mg I; 100 mg Mn; 0.5 mg Se; and 150 mg Zn.
[8]Dry matter basis. CP = crude protein; ADF = acid detergent fiber; and NDF = neutral detergent fiber.
[9]ND = none detected.

Table 2. Effect of mineral supplementation fed from -113 to 150 ± 16 d relative to parturition on primiparous beef heifer growth performance.

	Treatment[1]							
	0.15% S		0.55% S			Contrast		
Item[2]	No added Cu	Added Cu	No added Cu	Added Cu	SEM	Cu	S	Cu × S
Initial paddocks (heifers)	3 (9)	3 (9)	3 (9)	3 (9)				
Final paddocks (heifers)	3 (6)	3 (7)	3 (7)	3 (9)				
BW, kg								
Initial	404	403	387	400	19.2	0.67	0.47	0.62
Parturition	471	467	438	467	19.0	0.52	0.42	0.42
Final	421	435	393	403	19.2	0.57	0.29	0.63
BW change, kg								
Initial to parturition	66	64	56	67	9.7	0.65	0.71	0.50
Parturition to final	-51	-26	-46	-62	14.3	0.77	0.30	0.19
ADG,[3] kg/d								
Prepartum	0.59	0.57	0.49	0.59	0.09	0.65	0.71	0.50
Postpartum	-0.34	-0.18	-0.31	-0.42	0.10	0.77	0.30	0.19

Table 2 (Cont.)

	Treatment[1]							
	0.15% S		0.55% S			Contrast		
Item	No added Cu	Added Cu	No added Cu	Added Cu	SEM	Cu	S	Cu × S
BCS[4]								
Initial, No. (%)						0.63	0.38	0.82
5	4 (57)	4 (44)	3 (37)	3 (33)				
6	3 (43)	5 (56)	5 (63)	6 (68)				
Parturition, No. (%)						0.42	0.70	0.70
5	5 (71)	7 (78)	4 (57)	7 (78)				
6	2 (29)	2 (22)	3 (43)	2 (22)				
Final, No. (%)						1.00	0.98	1.00
4	0 (0)	0 (0)	2 (29)	4 (44)				
5	6 (100)	7 (100)	5 (71)	5 (56)				

[1]No added Cu provided 6 mg Cu/kg of DM. Added Cu provided 12 to 14 mg Cu/kg of DM.
[2]BW = body weight; ADG = average daily gain; and BCS = body condition score.
[3]Average daily gain was determined from BW collected prepartum (d -113, -85, -57, and -29 ± 16 relative to parturition) and postpartum (d 56, 85, 113 and 150 ± 16 relative to parturition).
[4]1 = emaciated to 9 = obese (Wagner et al., 1988).

Table 3. Effect of mineral supplementation fed to primiparous beef heifers from -113 to 150 ± 16 d relative to parturition on progeny growth performance.

	Treatment[1]							
	0.15% S		0.55% S			Contrast		
Item[2]	No added Cu	Added Cu	No added Cu	Added Cu	SEM	Cu	S	Cu × S
Paddocks (progeny)	3 (6)	3 (7)	3 (7)	3 (9)				
BW, kg								
Birth	28	33	33	31	1.6	0.50	0.47	0.09
Final	131	156	138	150	12.0	0.16	0.97	0.63
ADG,[3] kg/d	0.69	0.83	0.70	0.80	0.07	0.13	0.87	0.74
Calving ease,[4] No. (%)						1.00	1.00	1.00
1	7 (100)	8 (89)	7 (100)	9 (100)				
3	0 (0)	1 (11)	0 (0)	0 (0)				
Calf vigor,[5] No. (%)						1.00	1.00	1.00
1	7 (100)	9 (100)	7 (100)	9 (100)				
Agility score,[6] No. (%)						1.00	1.00	1.00
1	7 (100)	9 (100)	7 (100)	9 (100)				

Table 3 (Cont.)

[1]No added Cu provided 6 mg Cu/kg of DM. Added Cu provided 12 to 14 mg Cu/kg of DM.

[2]BW = body weight and ADG = average daily gain.

[3]From d 0 to 150 ± 6 relative to birth.

[4]1 = unassisted birth; 2 = minor assistance; 3 = mechanical assistance; 4 = cesarean-section; and 5 = abnormal presentation (Vandervelde et al., 1990).

[5]1 = alert and active; 2 = alert; 3 = appears healthy, but somewhat listless; 4 = listless; and 5 = listless and unresponsive (Vandervelde et al., 1990).

[6]1 = moves well and correct posture; 2 = moves showing slight stiffness in legs; 3 = significant stiffness in gate; 4 = significant stiffness in gate and slight arch in the topline; and 5 = significant stiffness in gate and arch in topline (Vandervelde et al., 1990).

Table 4. Least square means for the effects of mineral supplementation fed from -113 to 150 ± 16 d relative to parturition on primiparous beef heifer plasma and serum mineral status and ceruloplasmin activity.

	Treatment[1]							
	0.15% S		0.55% S			Contrast		
Item[2]	No added Cu	Added Cu	No added Cu	Added Cu	SEM	Cu	S	Cu × S
Plasma S,[3] mg/L								
Initial	42.9[a]	40.2[a]	41.3[a]	37.3[a]	1.3	0.02	0.12	0.60
Prepartum	32.5[b]	34.0[b]	34.1[b]	36.1[a]	1.2	0.20	0.17	0.86
Parturition	36.8[b]	36.1[b]	37.9[b]	38.6[a]	2.6	1.00	0.50	0.78
Postpartum	29.6[c]	28.8[c]	32.8[c]	33.6[b]	1.5	0.98	0.03	0.61
Plasma Cu, mg/L								
Initial	0.54	0.51[a]	0.51[a]	0.50	0.02	0.31	0.41	0.50
Prepartum	0.51	0.52[a]	0.44[b]	0.49	0.02	0.22	0.03	0.29
Parturition	0.56	0.61[b]	0.42[b]	0.51	0.04	0.14	0.02	0.59
Postpartum	0.54	0.53[a]	0.39[b]	0.47	0.04	0.42	0.07	0.33
Plasma Zn,[3] mg/L								
Initial	0.92[a]	0.94[a]	0.86[a]	0.99[a]	0.04	0.06	0.94	0.19

Table 4 (Cont.)

	Treatment[1]							
	0.15% S		0.55% S			Contrast		
Item[2]	No added Cu	Added Cu	No added Cu	Added Cu	SEM	Cu	S	Cu × S
Prepartum	0.82[a,b]	0.85[b]	0.85[a]	0.93[a]	0.07	0.45	0.47	0.74
Parturition	0.55[c]	0.53[c]	0.61[b]	0.72[b]	0.04	0.29	0.04	0.07
Postpartum	0.78[b]	0.74[d]	0.71[b]	0.76[b]	0.04	0.91	0.46	0.31
Serum Se,[3] mg/L								
Initial	0.17[a]	0.19[a]	0.18	0.17	0.01	0.34	0.97	0.09
Postpartum	0.23[b]	0.22[b]	0.18	0.20	0.03	0.91	0.21	0.61
Ceruloplasmin activity,[3] mg/dL								
Initial	37.9[a]	33.6[a]	40.2[a]	34.3[a]	1.9	0.08	0.46	0.69
Prepartum	42.8[a]	44.3[b]	31.8[b]	37.8[a]	2.0	0.11	0.04	0.30
Parturition	37.7[a]	48.8[c]	28.2[b]	35.7[a]	4.0	0.02	0.05	0.66
Postpartum	49.7[b]	47.1[c]	37.1[a]	38.8[b]	3.0	0.89	0.02	0.50

[1]No added Cu provided 6 mg Cu/kg of DM. Added Cu provided 12 to 14 mg Cu/kg of DM.
[2]Plasma mineral concentrations and ceruloplasmin activity were determined from blood samples collected initially (d -113 ± 16 relative to parturition), prepartum (d -85, -57, and -29 ± 16 relative to parturition), parturition, and postpartum (d 56, 85, 113 and 150

Table 4 (Cont.)

± 16 relative to parturition). Serum Se concentrations were determined from blood samples collected initially (d -113 ± 16 relative to parturition) and postpartum (d 150 ± 16 relative to parturition).

[3]Analyses conducted using initial values as a covariate.

[a-d]Means with no common superscripts in the dietary treatment and mineral differ ($P \leq 0.10$).

Table 5. Least square means for the effects of mineral supplementation fed from -113 to 150 ± 16 d relative to parturition on primiparous beef heifer liver mineral status (dry matter basis).

	Treatment[1]							
	0.15% S		0.55% S			Contrast		
Item[2]	No added Cu	Added Cu	No added Cu	Added Cu	SEM	Cu	S	Cu × S
S, %								
Initial	0.72	0.88	0.81	0.79[a]	0.06	0.29	1.00	0.17
Prepartum	0.75	0.71	0.66	0.71[a]	0.04	0.97	0.27	0.28
Postpartum	0.93	0.88	0.82	1.15[b]	0.29	0.65	0.79	0.54
Cu, mg/kg								
Initial	148	163	144[a]	146[a]	30	0.79	0.73	0.83
Prepartum	147	157	103[a,b]	125[a,b]	38	0.68	0.34	0.88
Postpartum	87	163	46[b]	66[b]	18	0.08	0.06	0.15
Zn, mg/kg								
Initial	166[a,b]	201	183	177	28	0.62	0.92	0.49
Prepartum	213[a]	174	238	183	16	0.03	0.32	0.63
Postpartum	122[b]	194	209	166	25	0.58	0.27	0.09

Table 5 (Cont.)

	Treatment[1]							
	0.15% S		0.55% S			Contrast		
Item[2]	No added Cu	Added Cu	No added Cu	Added Cu	SEM	Cu	S	Cu × S
Mo, mg/kg								
Initial	ND[4]	ND	ND	ND				
Prepartum	0.94	0.74	1.28	0.81	0.18	0.07	0.29	0.47
Postpartum	0.95	1.13	1.40	1.09	0.22	0.78	0.38	0.29

[1]No added Cu provided 6 mg Cu/kg of DM. Added Cu provided 12 to 14 mg Cu/kg of DM.
[2]Liver mineral concentrations were determined from liver samples collected initially (d -113 ± 16 relative to parturition), prepartum (d -57 ± 16 relative to parturition), and postpartum (56 and 113 ± 16 relative to parturition).
[4]ND = none detected.
[a,b]Means with no common superscripts in the same column and mineral differ ($P \leq 0.10$).

Table 6. Least square means for the effects of mineral supplementation fed to primiparous beef heifers from -113 to 150 ± 16 d relative to parturition on progeny plasma and serum mineral status and ceruloplasmin activity.

	Treatment[1]							
	0.15% S		0.55% S			Contrast		
Item[2]	No added Cu	Added Cu	No added Cu	Added Cu	SEM	Cu	S	Cu × S
Plasma S,[3] mg/L								
Birth	47.6[a]	43.9[a]	46.8[a]	39.0[a]	2.3	0.04	0.26	0.41
Preweaning	29.5[b]	28.0[b]	28.6[b]	28.7[b]	0.8	0.43	0.93	0.37
Plasma Cu, mg/L								
Birth	0.10[a]	0.11[a]	0.11[a]	0.10[a]	0.02	0.70	0.99	0.57
Preweaning	0.38[b]	0.41[b]	0.38[b]	0.38[b]	0.02	0.35	0.33	0.44
Plasma Zn, mg/L								
Birth	0.40	0.41	0.51	0.37[a]	0.07	0.39	0.60	0.35
Preweaning	0.63	0.65	0.68	0.62[b]	0.03	0.53	0.72	0.18
Serum Se, mg/L	0.13	0.14	0.09	0.09	0.02	0.80	0.04	0.74
Ceruloplasmin activity, mg/dL								
Birth	20.3[a]	18.7[a]	18.4[a]	17.7[a]	1.2	0.40	0.27	0.71

Table 6 (Cont.)

	Treatment[1]							
	0.15% S		0.55% S			Contrast		
Item[2]	No added Cu	Added Cu	No added Cu	Added Cu	SEM	Cu	S	Cu × S
Preweaning	38.9[b]	41.4[b]	38.6[b]	38.6[b]	1.9	0.53	0.43	0.54

[1]No added Cu provided 6 mg Cu/kg of DM. Added Cu provided 12 to 14 mg Cu/kg of DM.
[2]Plasma mineral concentrations and ceruloplasmin activity were determined from blood samples collected at birth and preweaning (d 31, 59, 86, 115, 141, and 150 ± 6 relative to birth). Serum Se concentrations were determined from blood samples collected d 150 ± 6 relative to birth.
[3]Analyses conducted using birth values as a covariate.
[a,b]Means with no common superscripts in the same column and mineral differ ($P \leq 0.10$).

Table 7. Least square means for the effects of mineral supplementation fed to primiparous beef heifers from -113 to 150 ± 16 d relative to parturition on progeny liver mineral status (dry matter basis).

	Treatment[1]							
	0.15% S		0.55% S			Contrast		
Item[2]	No added Cu	Added Cu	No added Cu	Added Cu	SEM	Cu	S	Cu × S
S, %								
Initial	0.94[a]	0.82[a]	0.98[a]	0.96[a]	0.23	0.75	0.69	0.84
Preweaning	0.37[b]	0.37[b]	0.38[b]	0.42[b]	0.05	0.68	0.46	0.62
Cu, mg/kg								
Initial	151	167	144	154	24	0.36	0.13	0.17
Preweaning	171	164	135	144	21	0.98	0.97	0.98
Zn,[3] mg/kg								
Initial	51[a]	112	93	92	14	0.07	0.48	0.06
Preweaning	104[b]	106	96	85	16	0.80	0.41	0.70
Mo, mg/kg								
Initial	0.60	0.90	0.96	0.79	0.21	0.75	0.58	0.30
Preweaning	0.60	0.57	0.50	0.43	0.13	0.75	0.44	0.86

Table 7 (Cont.)

[1]No added Cu provided 6 mg Cu/kg of DM. Added Cu provided 12 to 14 mg Cu/kg of DM.

[2]Liver mineral concentrations were determined from liver samples collected initially (d 7 ± 6 relative to birth) and during preweaning d 59 and 115 ± 6 relative to birth).

[3]Analyses conducted using initial values as a covariate.

[a,b]Means with no common superscripts in the same column and mineral differ ($P \leq 0.10$).

Table 8. Effect of mineral supplementation fed from -113 to 150 ± 16 d relative to parturition on postpartum primiparous beef heifer reproductive performance.

	Treatment[1]						
	0.15% S		0.55% S		Contrast		
Item, No. (%)	No added Cu	Added Cu	No added Cu	Added Cu	Cu	S	Cu × S
Reproductive tract score[2]					0.04	0.95	0.96
2	0 (0)	0 (0)	1 (14)	0 (0)			
3	3 (50)	0 (0)	2 (29)	1 (11)			
4	0 (0)	2 (25)	0 (0)	1 (11)			
5	3 (50)	6 (75)	4 (57)	7 (78)			
Corpus luteum development	3/6 (50)	6/8 (75)	4/7 (57)	7/9 (78)	0.31	0.76	0.83
Synchronized estrus response[3]							
First synchronized period	3/6 (50)	5/8 (63)	2/7 (29)	5/9 (56)	0.66	0.81	0.97
Second synchronized period[4]	0/3 (0)	4/5 (80)	2/5 (40)	3/6 (50)	1.00	1.00	1.00
Third synchronized period[5]	1/3 (33)	0/1 (0)	1/3 (33)	1/4 (25)	1.00	1.00	1.00
Synchronized conception rate[6]	0/6 (0)	4/8 (50)	2/7 (29)	3/9 (33)	0.07	0.34	0.53

[1]No added Cu provided 6 mg Cu/kg of DM. Added Cu provided 12 to 14 mg Cu/kg of DM.

Table 8 (Cont.)

[2]1 = no palpable follicles, no tone; 2 = 8 mm follicles, no tone; 3 = 8 to 10 mm follicles, slight tone; 4 = >10 mm follicles, good tone; 5 = >10 mm follicles, good tone, erect corpus luteum present. Reproductive-tract scores assigned 7 d before estrus synchronization (Anderson et al., 1991).

[3]All heifers received a controlled internal drug release (CIDR; 1.38 mg progesterone; Pfizer Animal Health) device on d 76 ± 16 relative to parturition and CIDR removal 7 d later concurrent with an intramuscular injection of 25 mg of prostaglandin F2α (5 mL Lutalyse; dinoprost tromethamine; Zoetis).

[4]Heifers that failed to respond to first CIDR insertion and prostaglandin F2α injection that were synchronized 10 to 13 d later.

[5]Heifers that failed to respond to second CIDR insertion and prostaglandin F2α injection that were synchronized 10 to 13 d later.

[6]Proportion of heifer that became pregnant of those exhibiting estrus and inseminated during synchronized period within each treatment.

CHAPTER VI

CONCLUSION

Exposing growing-finishing steers to greater S diets via supplemental dietary S from Na_2SO_4 had limited impact on performance and physiological responses, carcass characteristics, and beef quality after aging. Steers fed HS tolerated greater S intake than the 0.50% of dietary DM suggested by NRC (2005) as the maximum tolerable concentration for high roughage diets when supplementing Na_2SO_4 as a dietary S source. Moreover, steers fed HS tolerated greater S intake than the 0.30% of dietary DM suggested by NRC (2005) as the maximum tolerable concentration for high concentrate diets when supplementing Na_2SO_4 as a dietary S source. The inclusion of additional dietary S from Na_2SO_4 in steer growing and finishing diets produced beef with greater CLA content without a concomitant decrease in RD shelf-life. The shift in FA profile suggests supplemental dietary S from Na_2SO_4 has the potential to positively influence ruminal BH to alter the healthfulness of beef. Exposing growing-finishing cattle to greater S diets via supplemental dietary S from Na_2SO_4 decreased plasma Cu concentrations in steers during the finishing phase; however, plasma Cu concentrations were within the normal range and did not approach concentrations indicative of deficiency. The effect of feeding Na_2SO_4 was also characterized by increased blood SHb concentrations, though the increase was unlikely to cause hypoxia. The absence of a detectable change in CytOx activity in liver and LM from steers fed HS suggests the inclusion of additional dietary S from Na_2SO_4 in steer growing and finishing diets did not influence oxidative metabolism.

Exposing primiparous beef heifers consuming Cu deficient diets to greater S diets via supplemental dietary S from Na_2SO_4 has the potential to impact heifer physiological responses. Supplementing dietary Cu up to 12 to 14 mg Cu/kg of dietary DM to overcome the effects of feeding 0.55% S of dietary DM to primiparous beef heifers pre- and postpartum did not affect

heifer or progeny growth performance responses. Moreover, progeny liver Cu concentrations were similar among treatments and indicative of adequate liver Cu status in cattle. However, based on deficient heifer plasma and liver Cu status, the potential for dysfunction exists when feeding 0.55% S of DM to primiparous beef heifers consuming Cu deficient diets pre- and postpartum, and the supplementation of dietary Cu greater than 12 to 14 mg Cu/kg of DM is necessary to prevent Cu status from falling to levels at or near deficiency.

APPENDIX

UNIVERSITY OF ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Beth Kegley

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: October 10, 2012

SUBJECT: IACUC Protocol **APPROVAL**
Expiration date : **October 7, 2015**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol **#13008- "How does long term excess dietary sulfur affect growth performance and mitochondrial activity of cattle and flavor, color, and shelf-life of beef after aging?"**, with the **condition** that if any adverse effects are observed as the result of the biopsy procedures that sampling be halted and that the incident be immediately reported to the IACUC so that the situation can be reviewed. You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes to the protocol during the research, please notify the IACUC in writing [via the Modification Request form] **prior** to initiating the changes. If the study period is expected to extend beyond **10-07-2015**, you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

Administration Building 210 • 1 University of Arkansas • Fayetteville, AR 72701-1201 • 479-575-4572
Fax: 479-575-3846 • http://vpred.uark.edu/199

UNIVERSITY OF ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Beth Kegley

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: November 12, 2013

SUBJECT: IACUC Protocol **APPROVAL**
Expiration date : **November 14, 2016**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol **14015- " Performance, blood characteristics, and immune response in beef calves suckling cows supplemented with excess dietary sulfur during pregnancy and lactation** ". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes to the protocol during the research, please notify the IACUC in writing [via the Modification Request form] **prior** to initiating the changes. If the study period is expected to extend beyond **11-14-2016** you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

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Fax: 479-575-3846 • http://vpred.uark.edu/199

UNIVERSITY OF ARKANSAS

Office of Research Compliance
Institutional Review Board

September 18, 2013

MEMORANDUM

TO:	Jamie Hawley Elizabeth Kegley Jason Apple Jeremy Powell
FROM:	Ro Windwalker IRB Coordinator
RE:	New Protocol Approval
IRB Protocol #:	13-09-105
Protocol Title:	*How Does Long Term Excess Dietary Sulfur Affect Beef after Aging?*
Review Type:	☒ EXEMPT ☐ EXPEDITED ☐ FULL IRB
Approved Project Period:	Start Date: 09/18/2013 Expiration Date: 09/17/2014

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (http://vpred.uark.edu/210.php). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

This protocol has been approved for 150 participants. If you wish to make *any* modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 210 Administration Building, 5-2208, or irb@uark.edu.

210 Administration Building • 1 University of Arkansas • Fayetteville, AR 72701
Voice (479) 575-2208 • Fax (479) 575-3846 • Email irb@uark.edu

Simulated Retail Display Ballot

Please observe and rate the samples.

Sample	Total color score[1]								Worst-point color score[2, 3]								Surface discoloration, %					
	Very bright red	Bright red	Dull red	Slightly dark red	Moderately dark red	Dark red to dark reddish tan	Tannish red	Tan to brown	Very bright red	Bright red	Dull red	Slightly dark red	Moderately dark red	Dark red to dark reddish tan	Tannish red	Tan to brown	No discoloration, 0%	Slight discoloration, 1 to 20%	Small discoloration, 21 to 40%	Modest discoloration, 41 to 60%	Moderate discoloration, 61 to 80%	Extensive discoloration, 81 to 100%
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6
	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
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	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○

[1]Panelists can record scores to the nearest 0.5 point.

[2]Worst-point color is about the size of a dime.

[3]Panelists can record scores to the nearest 0.5 point.

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UNIVERSITY OF ARKANSAS
DALE BUMPERS COLLEGE OF AGRICULTURAL, FOOD & LIFE SCIENCES

Department of Animal Science
AFLS B114
University of Arkansas
Fayetteville, AR 72701-1201

INFORMED CONSENT

Title: How does long term excess dietary sulfur affect beef flavor after aging?

Researchers: Jamie Hawley, M.S., Doctoral Student
Elizabeth Kegley, Ph.D., Faculty
Jason Apple, Ph.D., Faculty
Jeremy Powell, Ph.D., Faculty
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Administrator: Ro Windwalker, CIP
IRB Coordinator
Office of Research Compliance
ADMN 210
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(479) 575-2208
irb@uark.edu

Description: Consumers rate attributes related to the eating experience (i.e., flavor and tenderness) as essential when purchasing beef. In this study, you will be asked to evaluate sensory attributes (e.g., flavor and juiciness) of beef steaks.

Risks and benefits: The results of this study will be used to help reduce the impact of dietary sulfur on beef quality before consumers become dissatisfied. There are no anticipated risks to participating in the study.

Voluntary participation: Your participation in the research is completely voluntary. There are no college credits for participation.

Confidentiality: You will be assigned a code number and all information will be recorded anonymously. All information will be kept confidential to the extent allowed by law and University policy. Results from the research will be reported as aggregate data.

Right to withdraw: You are free to refuse to participate in the research and to withdraw from this study at any time. Your decision to withdraw will bring no negative consequences — no penalty to you.

Informed consent: I, _____(please print), have read the description, including the purpose of the study, the procedures to be used, the potential risks and side effects, the confidentiality, as well as the option to withdraw from the study at any time. Each of these items has been explained to me by the investigator. The investigator has answered all of my questions regarding the study, and I believe I understand what is involved. My signature below indicates that I freely agree to participate in this experimental study and that I have received a copy of this agreement from the investigator.

Signature _____ **Date** _____

If you have questions or concerns about this study, please contact one of the researchers listed above. For questions or concerns about your rights as a research participant, please contact the University's IRB Coordinator listed as "Administrator" above.

Consumer Sensory Evaluation Ballot

Please observe and taste this sample.

1. Please select the statement that best describes your impression of the **OVERALL TENDERNESS** of this product.								
Dislike extremely	Dislike very much	Moderately dislike	Dislike slightly	Neither like nor dislike	Like slightly	Moderately like	Like very much	Like extremely
○	○	○	○	○	○	○	○	○

2. Please select the statement that best describes your impression of the **JUICINESS** of this product.								
Dislike extremely	Dislike very much	Moderately dislike	Dislike slightly	Neither like nor dislike	Like slightly	Moderately like	Like very much	Like extremely
○	○	○	○	○	○	○	○	○

3. Please select the statement that best describes your impression of the **BEEF FLAVOR** of this product.								
Dislike extremely	Dislike very much	Moderately dislike	Dislike slightly	Neither like nor dislike	Like slightly	Moderately like	Like very much	Like extremely
○	○	○	○	○	○	○	○	○

4. Please select the statement that best describes your impression of the **OFF-FLAVOR INTENSITY** of this product.								
Dislike extremely	Dislike very much	Moderately dislike	Dislike slightly	Neither like nor dislike	Like slightly	Moderately like	Like very much	Like extremely
○	○	○	○	○	○	○	○	○

5. Considering the **OVERALL TENDERNESS** of the product, please select the statement that best describes your impression of this product.				
Very tough	Tough	Moderately tender	Tender	Very tender
○	○	○	○	○

6. Considering the intensity of the **OVERALL FLAVOR** of the product, please select the statement that best describes your impression of this product.				
Much too weak	A little too weak	Just about right	A little too strong	Much too strong
○	○	○	○	○

Consumer Sensory Evaluation Ballot (Cont.)

Please complete the following demographic information.

1. Please select your sex.	
◯ Male	◯ Female

2. Please select your age group.		
◯ 18-24 years old	◯ 25-34 years old	◯ 35-45 years old
◯ 46-54 years old	◯ 55-65 years old	◯ Over 65 years old

3. Please select the category that best represents your annual household income.	
◯ Under $15,000 per year	◯ $15,000 to $19,999 per year
◯ $20,000 to $29,999 per year	◯ $30,000 to $39,999 per year
◯ $40,000 to $49,999 per year	◯ $50,000 to $59,999 per year
◯ $60,000 to $69,999 per year	◯ $70,000 to $79,999 per year
◯ $80,000 to $89,999 per year	◯ $90,000 to $99,999 per year
◯ More than $100,000 per year	